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ATG GTA CGT AGC TCC TCT CGC ACT CCG TCC GAT AAG CCG GTT GCT  
 M V R S S S R T P S D K P V A  
  
 CAT GTA GTT GCT AAC CCT CAG GCA GAA GGT CAG CTG CAG TGG CTG  
 H V V A N P Q A E G Q L Q W L  
  
 AAC CGT CGC GCT AAC GCC CTG CTG GCA AAC GGC GTT GAG CTC CGT  
 N R R A N A L L A N G V E L R  
  
 GAT AAC CAG CTC GTG GTA CCT TCT GAA GGT CTG TAC CTG ATC TAT  
 D N Q L V V P S E G L Y L I Y  
  
 TCT CAA GTA CTG TTC AAG GGT CAG GGC TGC CCG TCG ACT CAT GTT  
 S Q V L F K G Q G C P S T H V  
  
 CTG CTG ACT CAC ACC ATC AGC CGT ATT GCT GTA TCT TAC CAG ACC  
 L L T H T I S R I A V S Y Q T  
  
 AAA GTT AAC CTG CTG AGC GCT ATC AAG TCT CCG TGC CAG CGT GAA  
 K V N L L S A I K S P C Q R E  
  
 ACT CCC GAG GGT GCA GAA GCG AAA CCA TGG TAT GAA CCG ATC TAC  
 T P E G A E A K P W Y E P I Y  
  
 CTG GGT GGC GTA TTT CAA CTG GAG AAA GGT GAC CGT CTG TCC GCA  
 L G G V F Q L E K G D R L S A  
  
 GAA ATC AAC CGT CCT GAC TAT CTA GAT TTC GCT GAA TCT GGC CAG  
 E I N R P D Y L D F A E S G Q  
  
 GTG TAC TTC GGT ATT ATC GCA CTG TAA  
 V Y F G I I A L \*

**Derivation of the VNP20009(*serC*<sup>-</sup>) strain.**

**pCR2.1*serC***

(*serC* cloned by pcr into pCR2.1)

↓

Cla1+Eco47III restriction  
Mung bean nuclease degradation  
Religation  
Transformation into DH5 $\alpha$

**pCR2.1Δ*serC***

(*serC* deletion cloned by pcr into pCR2.1)

↓

Sac1+Xho1 restriction  
Isolation of 680 bp Δ*serC* gene  
Ligation into pCVD442  
Transformation into SM10 cells

**pCVD442Δ*serC***

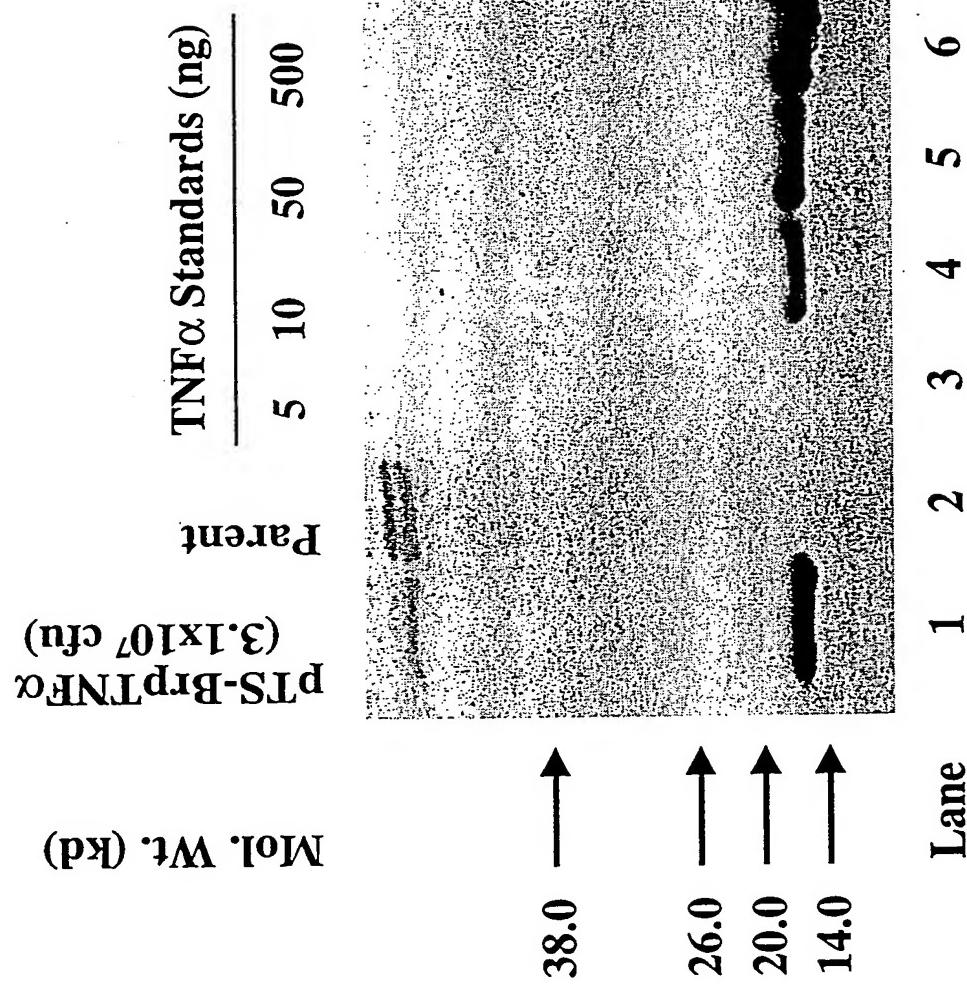
(*serC* deletion cloned into pCVD442 sucrase vector)

↓

SM10 bacteria mated with *S. typhimurium*  
strain 501 to form merodiploid  
*serC* deletion transduced into VNP20009 using  
P22 bacteriophage  
VNP20009(*serC*<sup>-</sup>) obtained by sucrose selection

**VNP20009 (*serC*<sup>-</sup>)**

**FIG. 2**

**Quantitation of TNF $\alpha$  expression by pTS-BrpTNF $\alpha$  Clone 2.****FIG. 3**

ATG AAA AAG ACA GCT ATC GCG ATT GCA GTG GCA CTG GCT GGT TTC  
 M K K T A I A I A V A L A G F  
 GCT ACC GTA GCG CAG GCC CAT ATG GTA CGT AGC TCC TCT CGC ACT  
 A T V A Q A H M V R S S S R T  
 CCG TCC GAT AAG CCG GTT GCT CAT GTA GTT GCT AAC CCT CAG GCA  
 P S D K P V A H V V A N P Q A  
 GAA GGT CAG CTG CAG TGG CTG AAC CGT CGC GCT AAC GCC CTG CTG  
 E G Q L Q W L N R R A N A L L  
 GCA AAC GGC GTT GAG CTC CGT GAT AAC CAG CTC GTG GTA CCT TCT  
 A N G V E L R D N Q L V V P S  
 GAA GGT CTG TAC CTG ATC TAT TCT CAA GTA CTG TTC AAG GGT CAG  
 E G L Y L I Y S Q V L F K G Q  
 GGC TGC CCG TCG ACT CAT GTT CTG CTG ACT CAC ACC ATC AGC CGT  
 G C P S T H V L L T H T I S R  
 ATT GCT GTA TCT TAC CAG ACC AAA GTT AAC CTG CTG AGC GCT ATC  
 I A V S Y Q T K V N L L S A I  
 AAG TCT CCG TGC CAG CGT GAA ACT CCC GAG GGT GCA GAA GCG AAA  
 K S P C Q R E T P E G A E A K  
 CCA TGG TAT GAA CCG ATC TAC CTG GGT GGC GTA TTT CAA CTG GAG  
 P W Y E P I Y L G G V F Q L E  
 AAA GGT GAC CGT CTG TCC GCA GAA ATC AAC CGT CCT GAC TAT CTA  
 K G D R L S A E I N R P D Y L  
 GAT TTC GCT GAA TCT GGC CAG GTG TAC TTC GGT ATT ATC GCA CTG  
 D F A E S G Q V Y F G I I A L

TAA  
\*

Expression and processing of a *trc* promoter-driven *ompA-TRAIL* fusion gene product in JM109 bacteria.

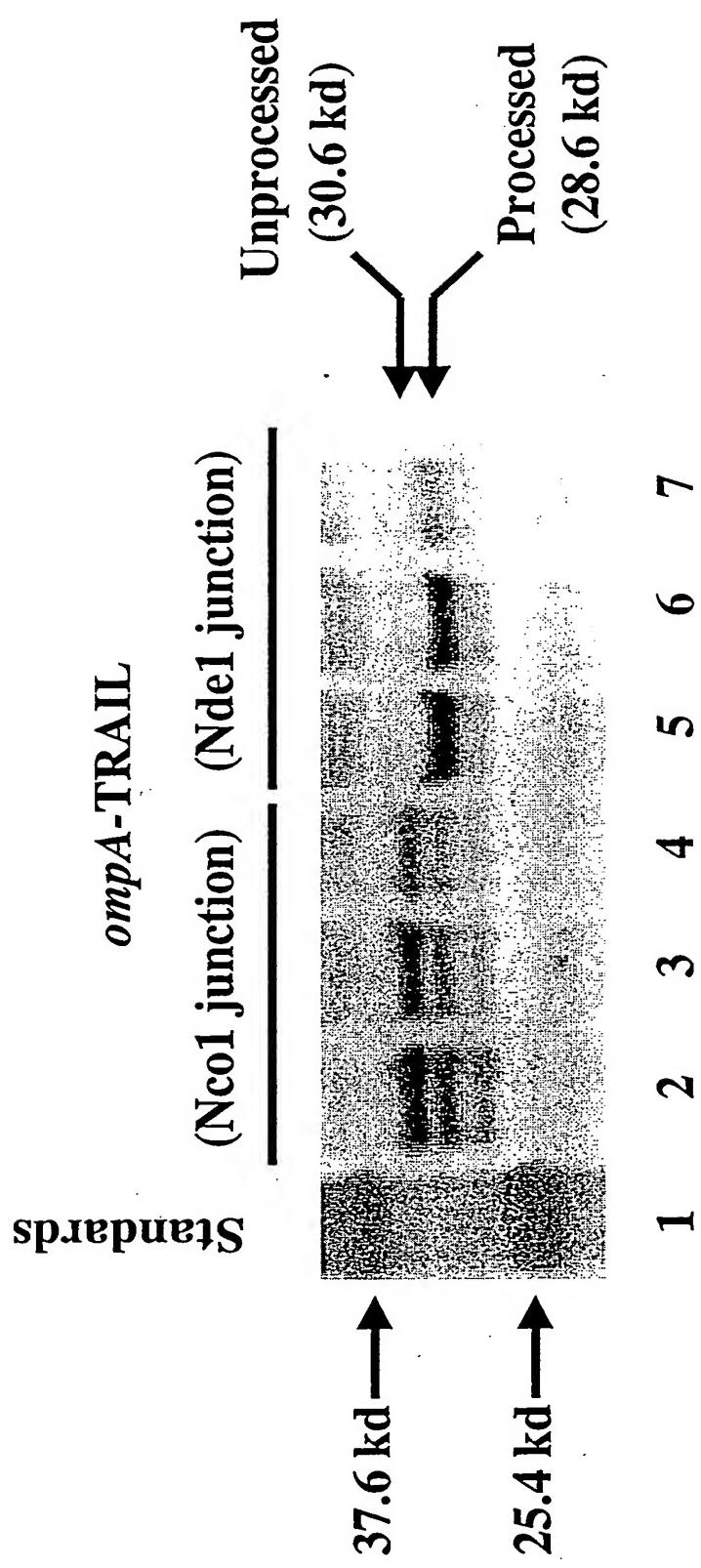


FIG. 5

ATG AAA AAG ACA GCT ATC GCG ATT GCA GTG GCA CTG GCT GGT TTC  
 M K K T A I A I A V A L A G F  
  
 GCT ACC GTA GCG CAG GCC CAT ATG GCT AAC GAG CTG AAG CAG ATG  
 A T V A Q A H M A N E L K Q M  
  
 CAG GAC AAG TAC TCC AAA AGT GGC ATT GCT TGT TTC TTA AAA GAA  
 Q D K Y S K S G I A C F L K E  
  
 GAT GAC AGT TAT TGG GAC CCC AAT GAC GAA GAG AGT ATG AAC AGC  
 D D S Y W D P N D E E S M N S  
  
 CCC TGC TGG CAA GTC AAG TGG CAA CTC CGT CAG CTC GTT AGA AAG  
 P C W Q V K W Q L R Q L V R K  
  
 ATG ATT TTG AGA ACC TCT GAG GAA ACC ATT TCT ACA GTT CAA GAA  
 M I L R T S E E T I S T V Q E  
  
 AAG CAA CAA AAT ATT TCT CCC CTA GTG AGA GAA AGA GGT CCT CAG  
 K Q Q N I S P L V R E R G P Q  
  
 AGA GTA GCA GCT CAC ATA ACT GGG ACC AGA GGA AGA AGC AAC ACA  
 R V A A H I T G T R G R S N T  
  
 TTG TCT TCT CCA AAC TCC AAG AAT GAA AAG GCT CTG GGC CGC AAA  
 L S S P N S K N E K A L G R K  
  
 ATA AAC TCC TGG GAA TCA TCA AGG AGT GGG CAT TCA TTC CTG AGC  
 I N S W E S S R S G H S F L S  
  
 AAC TTG CAC TTG AGG AAT GGT GAA CTG GTC ATC CAT GAA AAA GGG  
 N L H L R N G E L V I H E K G  
  
 TTT TAC TAC ATC TAT TCC CAA ACA TAC TTT CGA TTT CAG GAG GAA  
 F Y Y I Y S Q T Y F R F Q E E  
  
 ATA AAA GAA AAC ACA AAG AAC GAC AAA CAA ATG GTC CAA TAT ATT  
 I K E N T K N D K Q M V Q Y I  
  
 TAC AAA TAC ACA AGT TAT CCT GAC CCT ATA TTG TTG ATG AAA AGT  
 Y K Y T S Y P D P I L L M K S  
  
 GCT AGA AAT AGT TGT TGG TCT AAA GAT GCA GAA TAT GGA CTC TAT  
 A R N S C W S K D A E Y G L Y  
  
 TCC ATC TAT CAA GGG GGA ATA TTT GAG CTT AAG GAA AAT GAC AGA  
 S I Y Q G G I F E L K E N D R  
  
 ATT TTT GTT TCT GTA ACA AAT GAG CAC TTG ATA GAC ATG GAC CAT  
 I F V S V T N E H L I D M D H  
  
 GAA GCC AGT TTT TTC GGG GCC TTT TTA GTT GGC TAA  
 E A S F F G A F L V G \*

Expression and processing of a *trc* promoter-driven *ompA-TNF $\alpha$*  fusion gene product in JM109 bacteria.

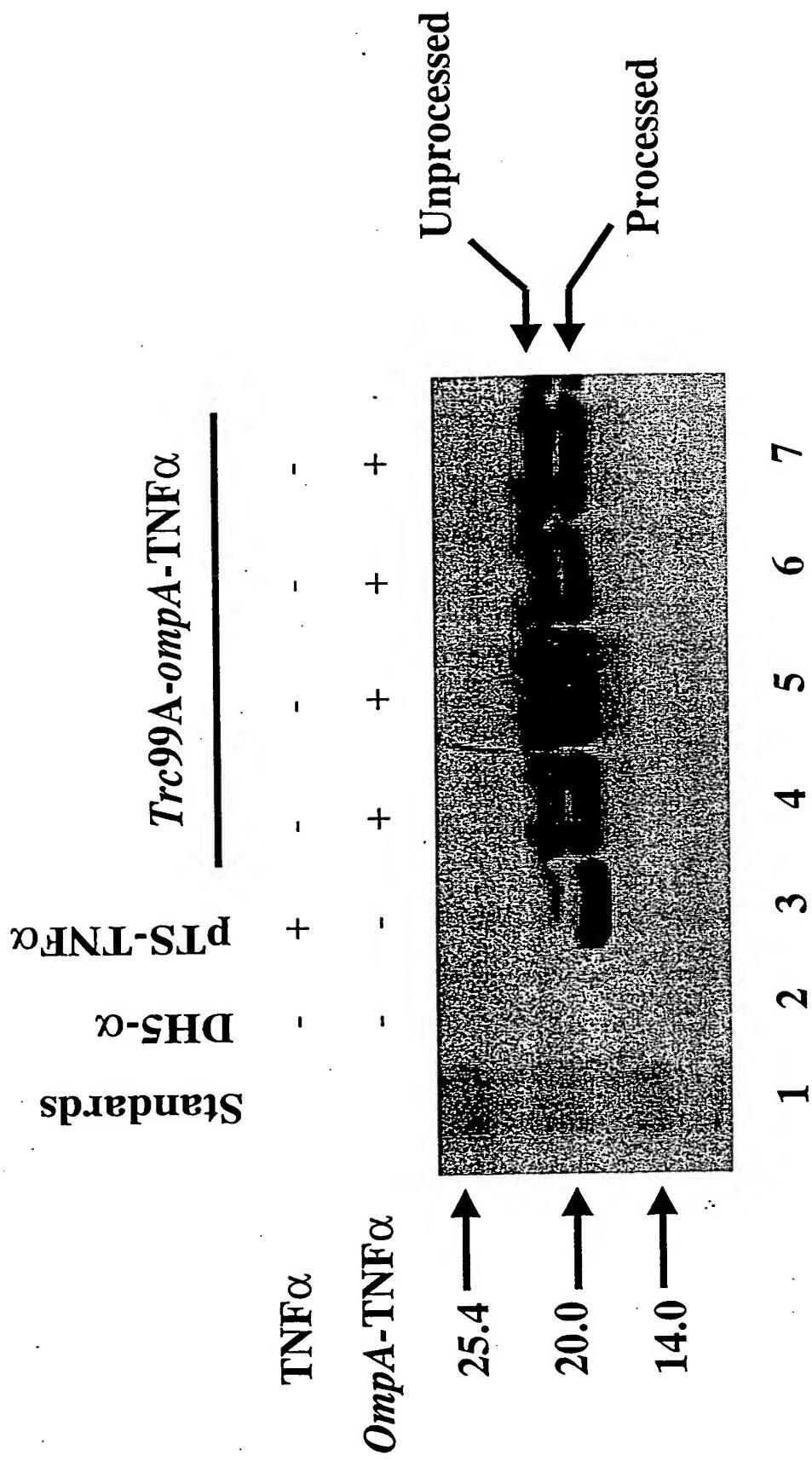


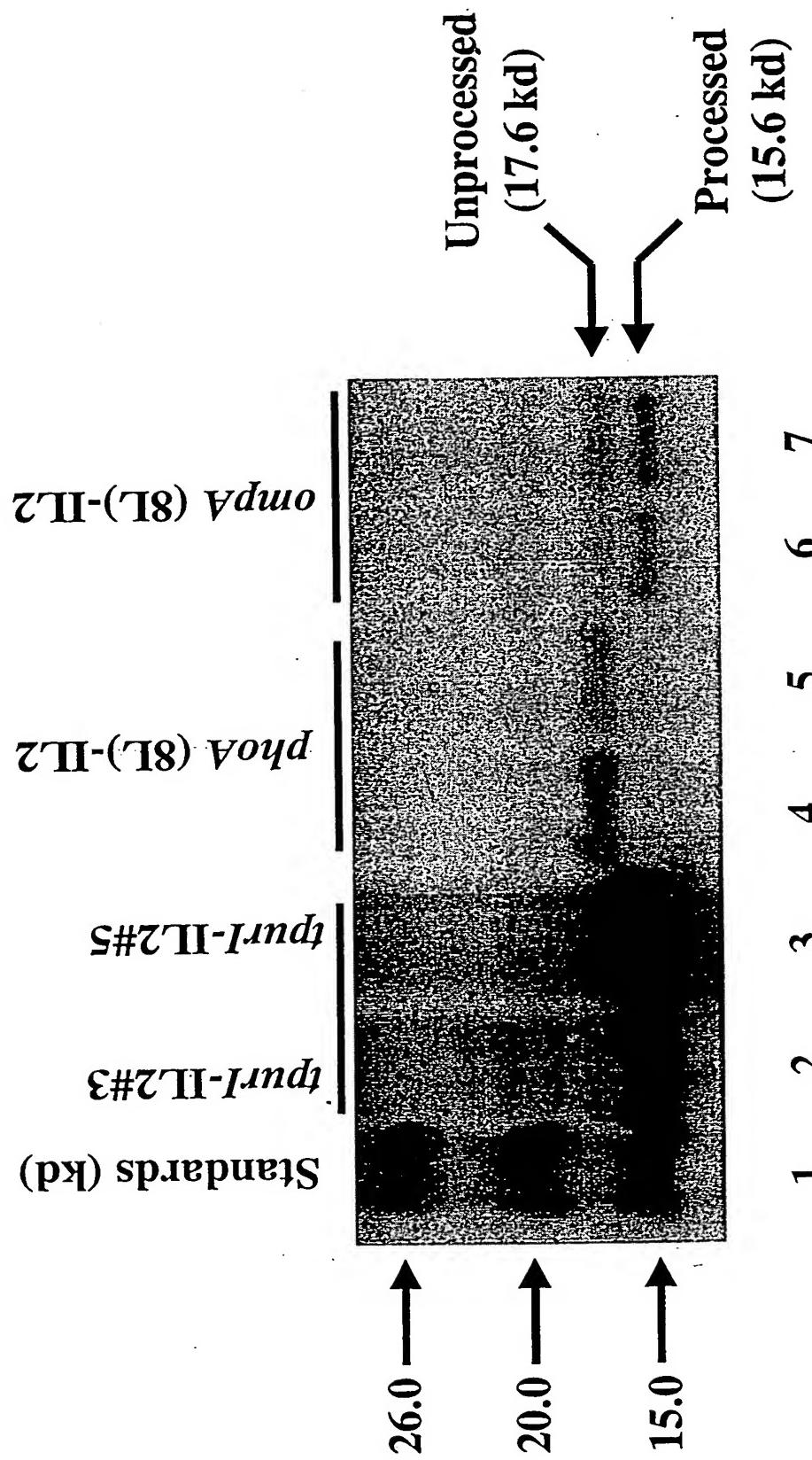
FIG. 7

ATG AAA AAG ACG GCT CTG GCG CTT CTG CTC TTG CTG TTA GCG CTG  
 M K K T A L A L L L L L A L  
  
 ACT AGT GTA GCG CAG GCC GCT CCT ACT AGC TCG AGC ACT AAG AAA  
 T S V A Q A A P T S S S T K K  
  
 ACT CAA CTG CAA TTG GAG CAT CTG CTG CTG GAT CTG CAG ATG ATT  
 T Q L Q L E H L L L D L Q M I  
  
 CTG AAT GGC ATC AAT AAC TAC AAG AAC CCT AAG CTG ACT CGC ATG  
 L N G I N N Y K N P K L T R M  
  
 CTG ACT TTC AAA TTC TAC ATG CCG AAA AAG GCT ACC GAG CTC AAA  
 L T F K F Y M P K K A T E L K  
  
 CAT CTC CAG TGC CTG GAA GAG GAA CTG AAG CCG CTG GAG GAA GTA  
 H L Q C L E E E L K P L E E V  
  
 CTT AAC CTG GCA CAG TCT AAG AAC TTC CAC CTG CGT CCG CGT GAC  
 L N L A Q S K N F H L R P R D  
  
 CTG ATC TCC AAC ATC AAT GTA ATC GTT CTT GAG CTG AAG GGA TCC  
 L I S N I N V I V L E L K G S  
  
 GAA ACC ACC TTC ATG TGC GAA TAC GCT GAC GAA ACC GCC ACC ATT  
 E T T F M C E Y A D E T A T I  
  
 GTG GAG TTC CTG AAC CGT TGG ATC ACC TTT GCC CAA TCG ATC ATT  
 V E F L N R W I T F A Q S I I  
  
 AGC ACG TTA ACT TAA  
 S T L T \*

FIG. 8

Periplasmic localization and processing of *ompA*-IL2 fusion proteins.

FIG. 9



ATG AAA CAG TCG ACT CTG GCG CTT CTG CTC TTG CTG TTA GCG CTG  
M K Q S T L A L L L L L L A L

ACT AGT GTG GCC AAA GCG GCT CCT ACT AGC TCG AGC ACT AAG AAA  
T S V A K A A P T S S S T K K

ACT CAA CTG CAA TTG GAG CAT CTG CTG GAT CTG CAG ATG ATT  
T Q L Q L E H L L L D L Q M I

CTG AAT GGC ATC AAT AAC TAC AAG AAC CCT AAG CTG ACT CGC ATG  
L N G I N N Y K N P K L T R M

CTG ACT TTC AAA TTC TAC ATG CCG AAA AAG GCT ACC GAG CTC AAA  
L T F K F Y M P K K A T E L K

CAT CTC CAG TGC CTG GAA GAG GAA CTG AAG CCG CTG GAG GAA GTA  
H L Q C L E E L K P L E E V

CTT AAC CTG GCA CAG TCT AAG AAC TTC CAC CTG CGT CCG CGT GAC  
L N L A Q S K N F H L R P R D

CTG ATC TCC AAC ATC AAT GTA ATC GTT CTT GAG CTG AAG GGA TCC  
L I S N I N V I V L E L K G S

GAA ACC ACC TTC ATG TGC GAA TAC GCT GAC GAA ACC GCC ACC ATT  
E T T F M C E Y A D E T A T I

GTG GAG TTC CTG AAC CGT TGG ATC ACC TTT GCC CAA TCG ATC ATT  
V E F L N R W I T F A Q S I I

AGC ACG TTA ACT TAA  
S T L T \*

Antitumor efficacy of pTS-BrpTNF $\alpha$  Clone 2 in a staged  
Colon 38 tumor model.

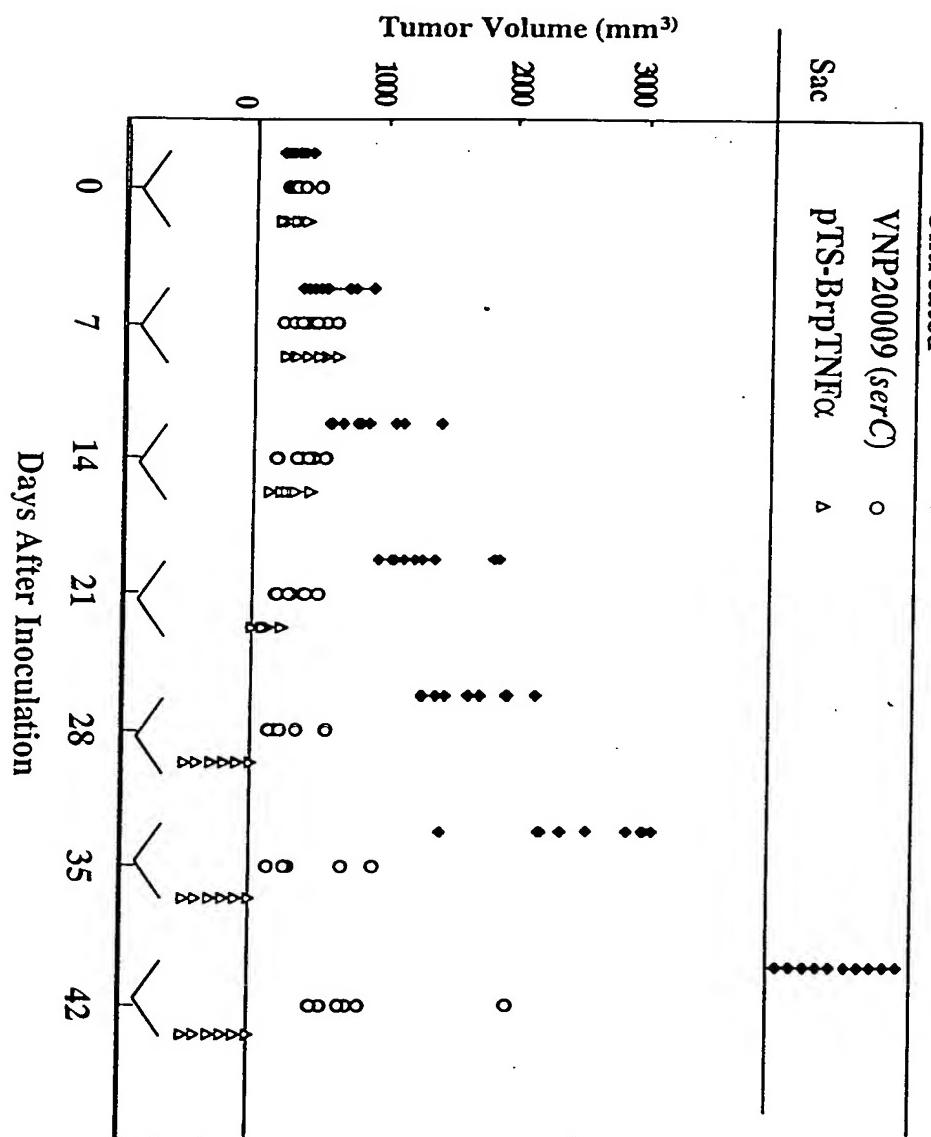


FIG. 11

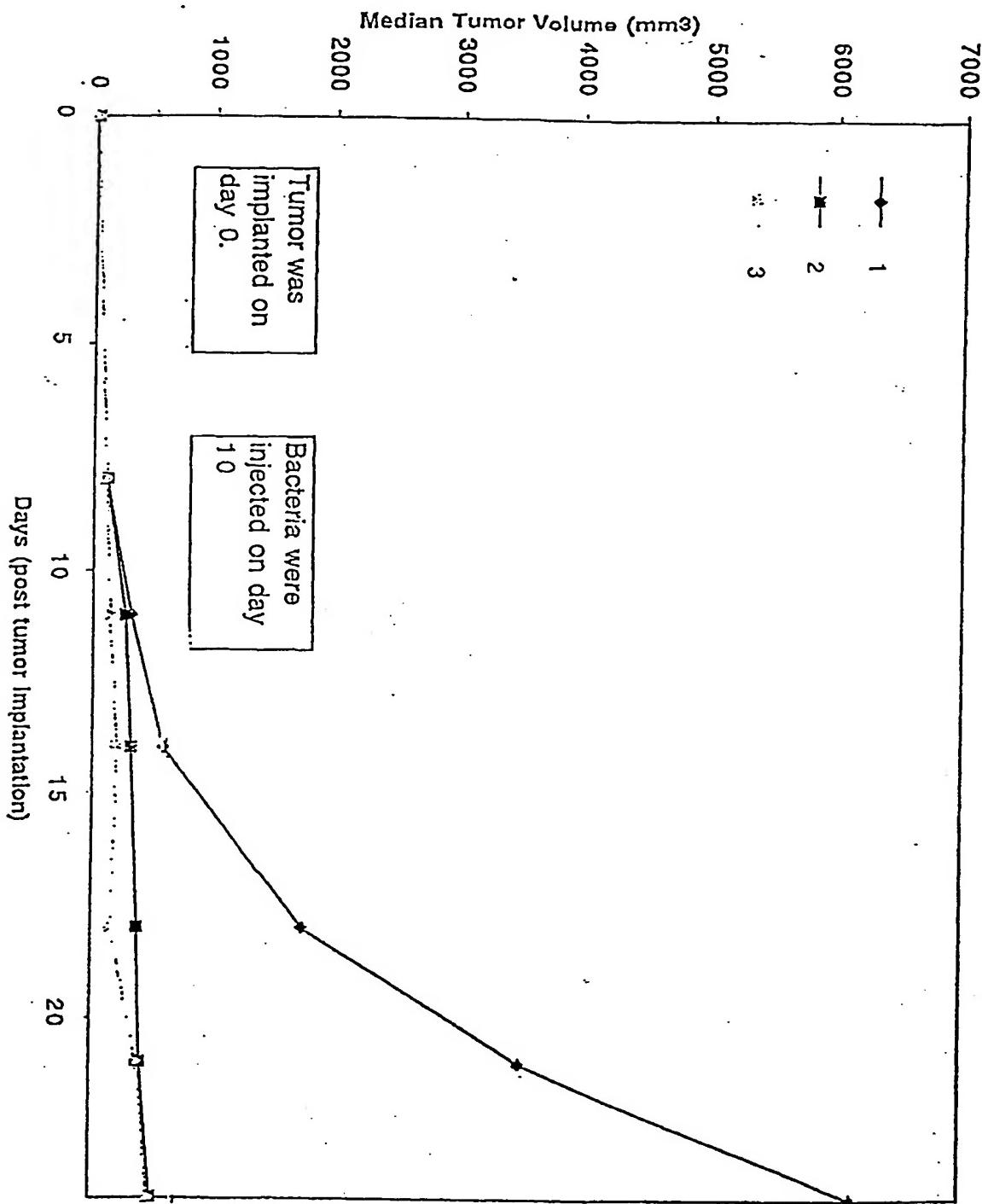


FIG. 12

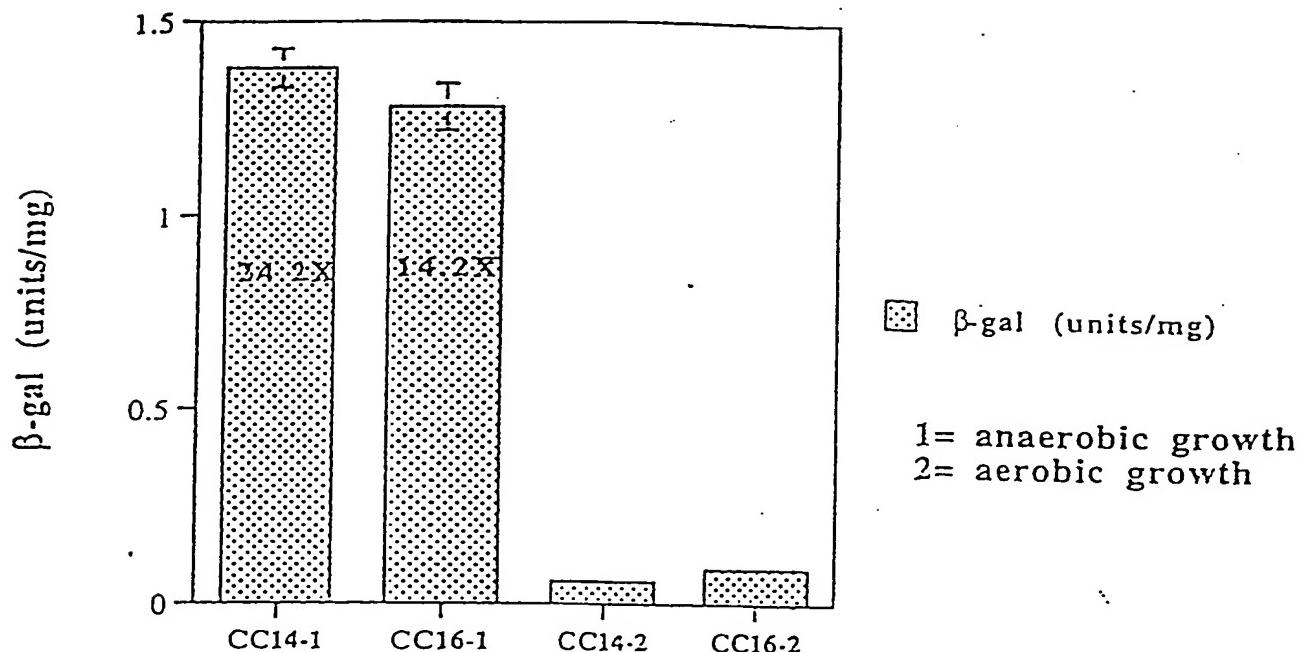
$\beta$ -gal activity in strains carrying pepT/bgal

FIG. 13A

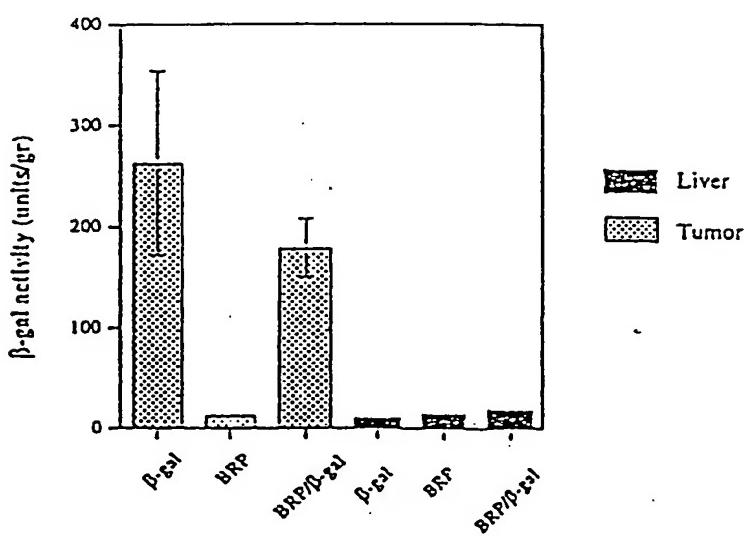
 $\beta$ -gal activity *in vivo*, pepT $\beta$ -gal  $\pm$  BRP

FIG. 13B

Expression of  $\beta$ -gal using  
TET promoter

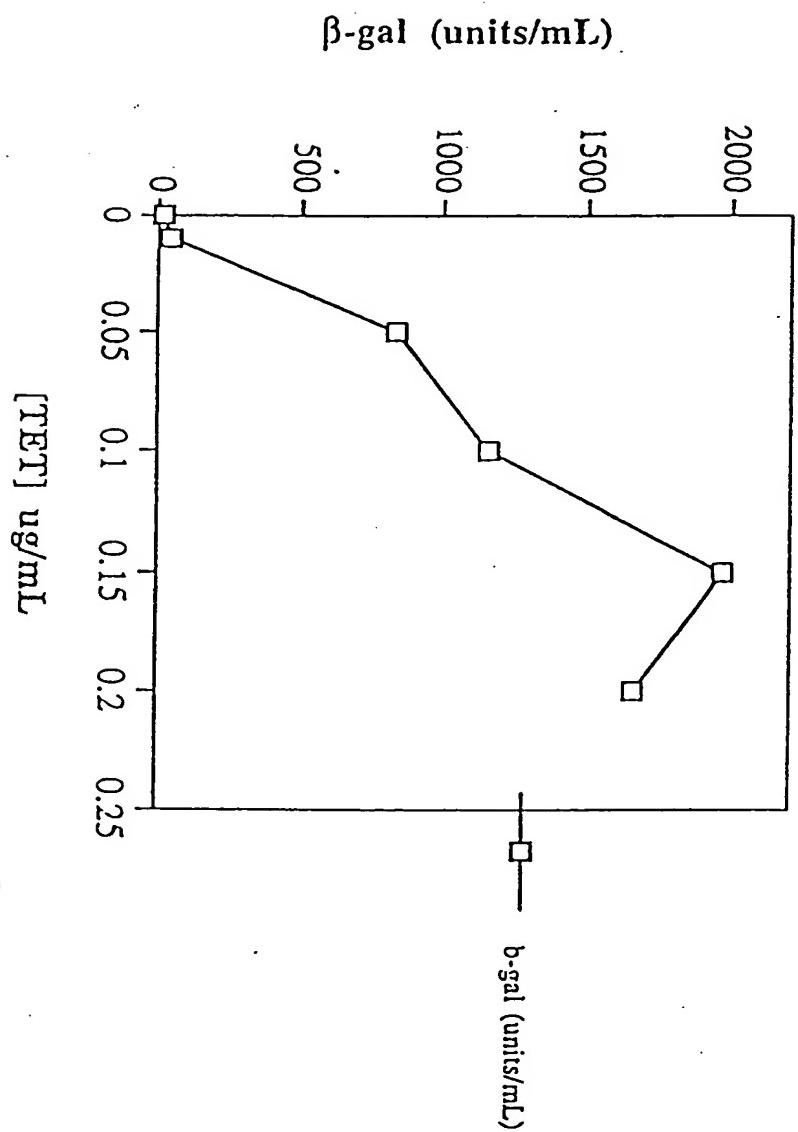
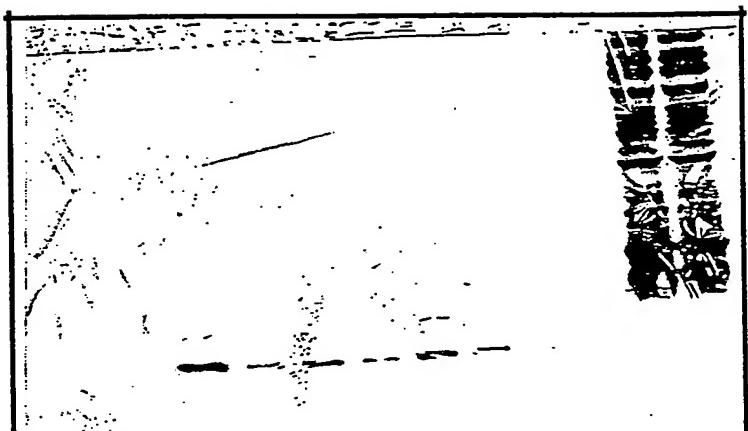


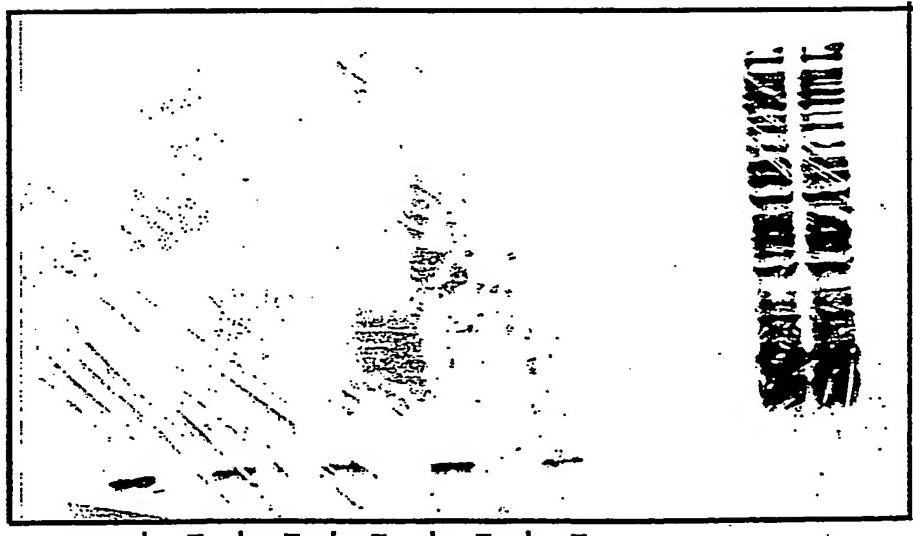
FIG. 14

**A.**

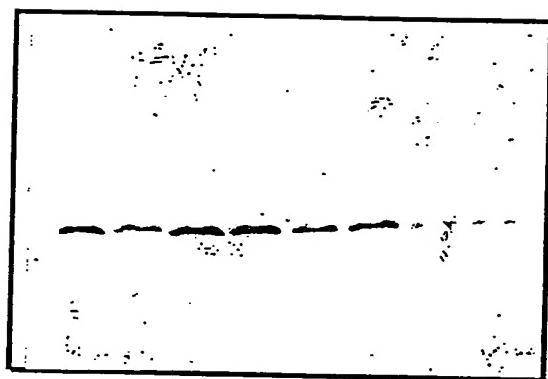
~25 kD →  
HexaHIS-endostatin

**B.**

~25 kD →  
HexaHIS-endostatin

**FIG. 15**

**~25kD** →  
**HexaHIS-endostatin**



**FIG. 16**

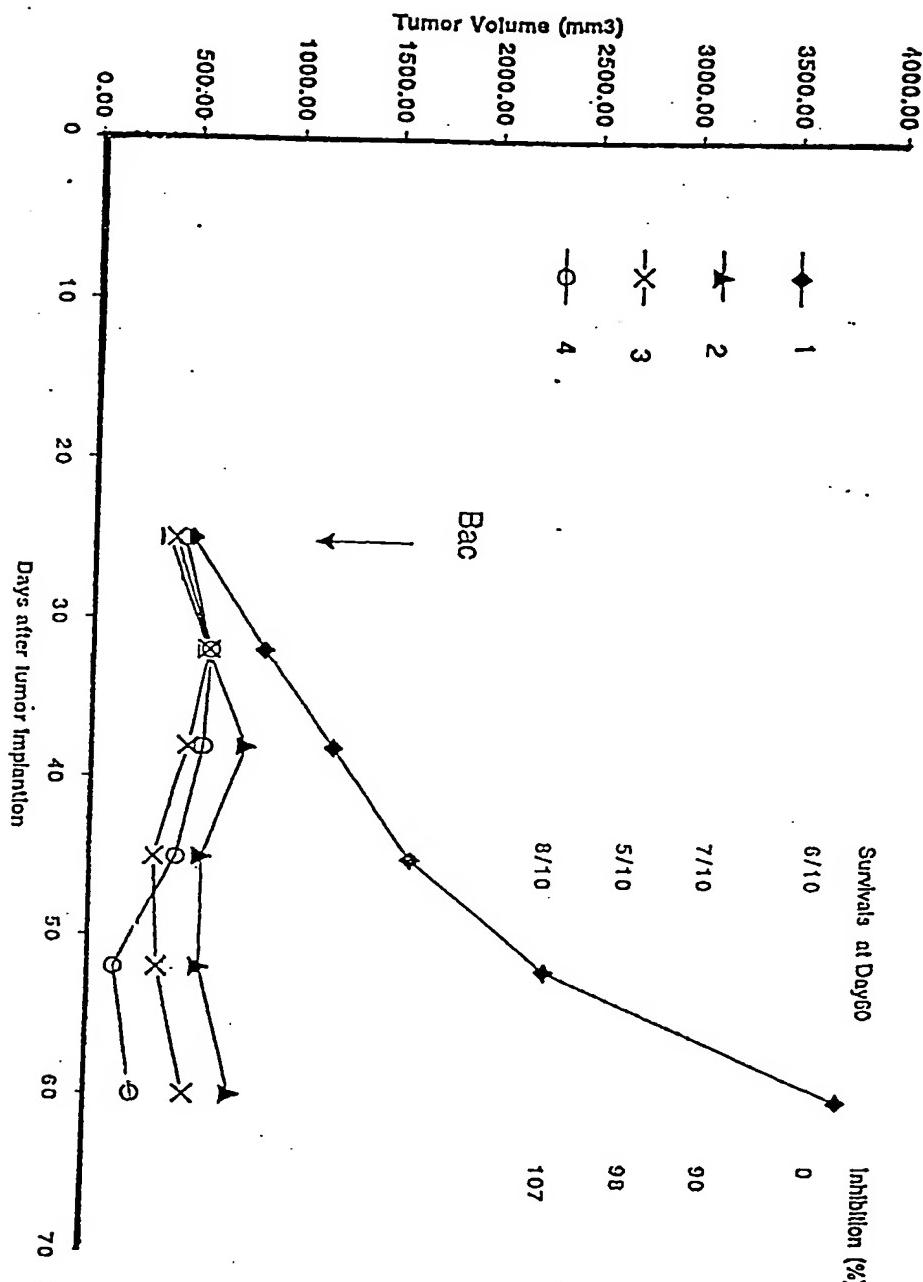


FIG. 17

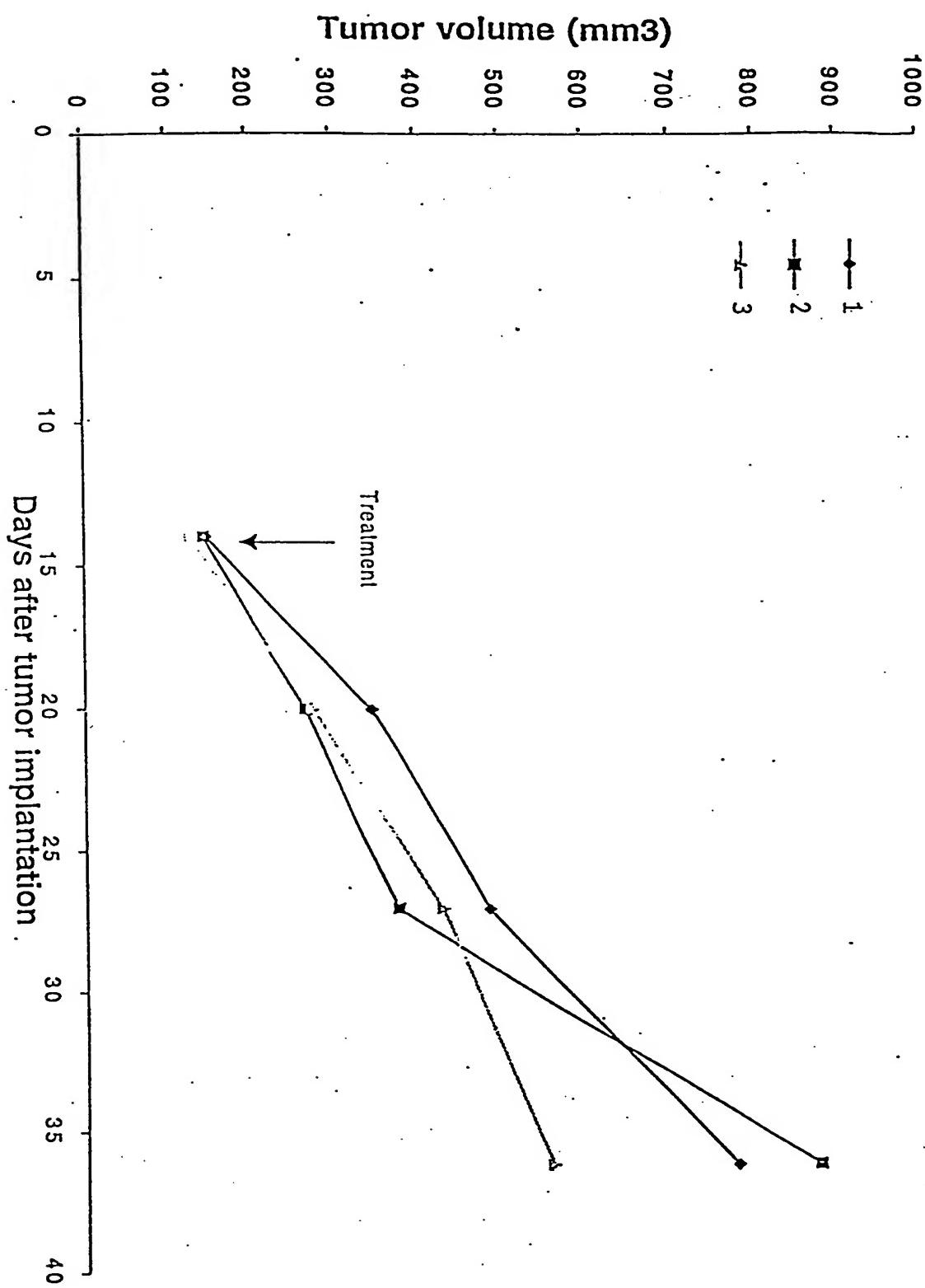
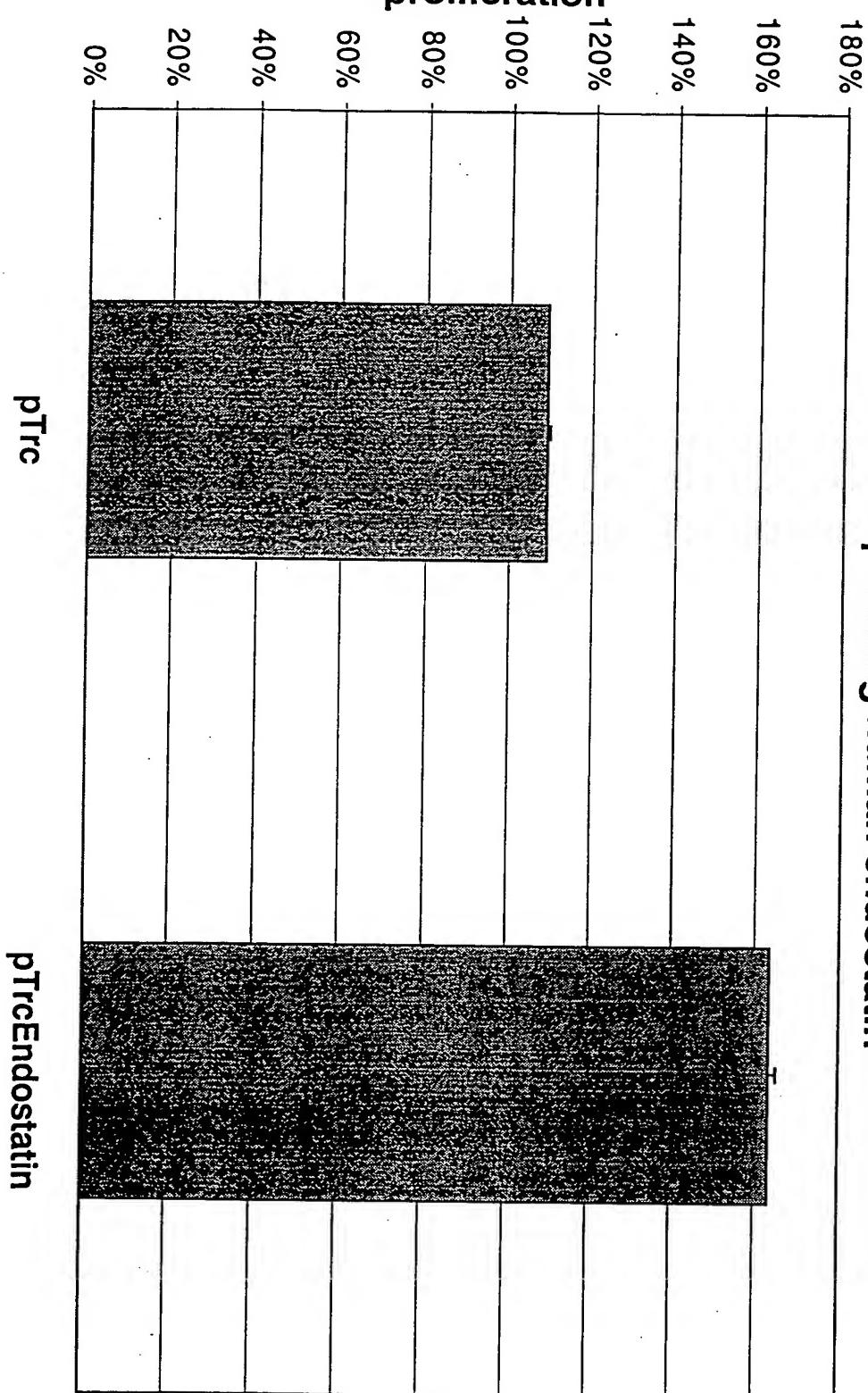
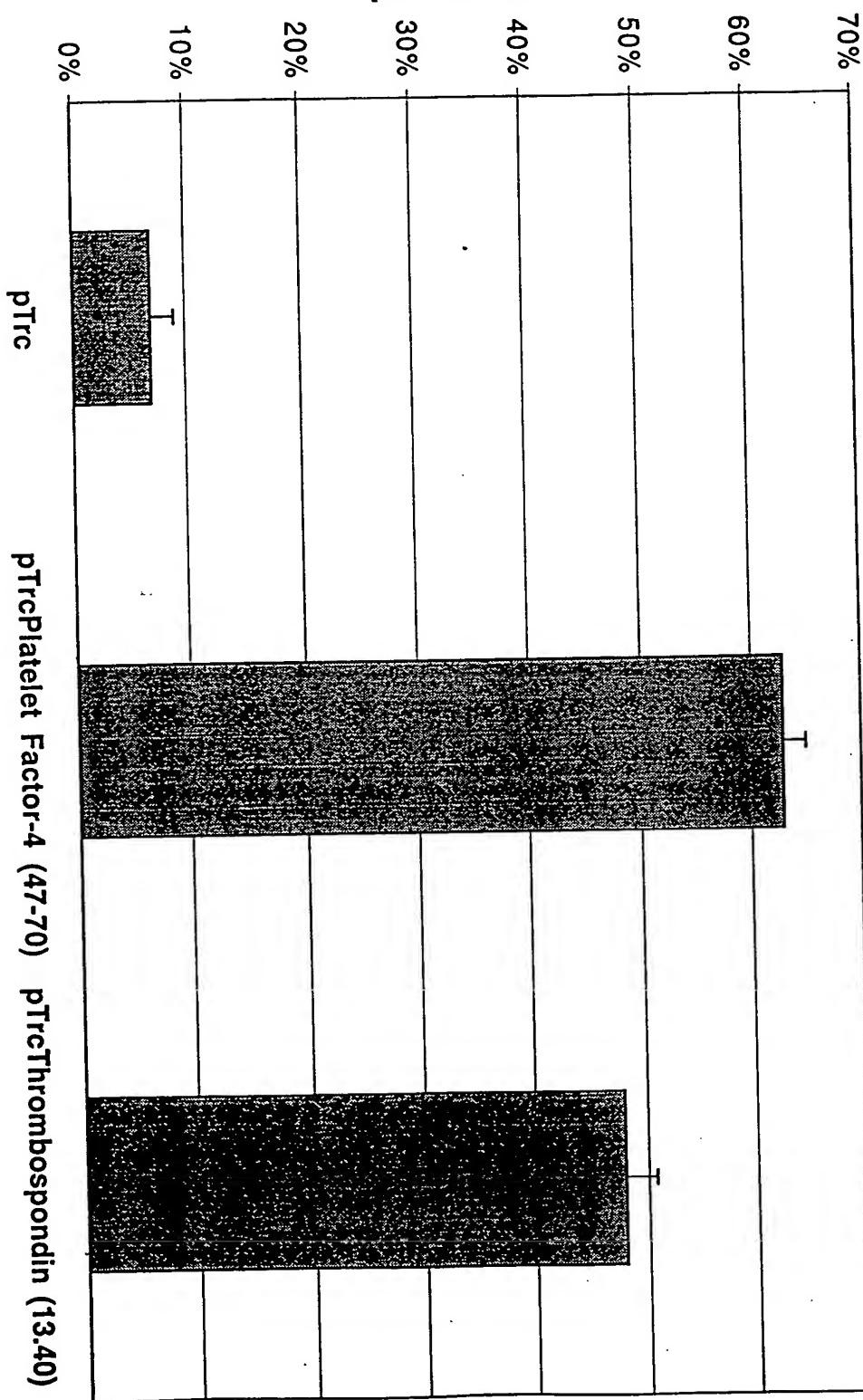


FIG. 18

**Inhibition of endothelial cell proliferation****FIG. 19**

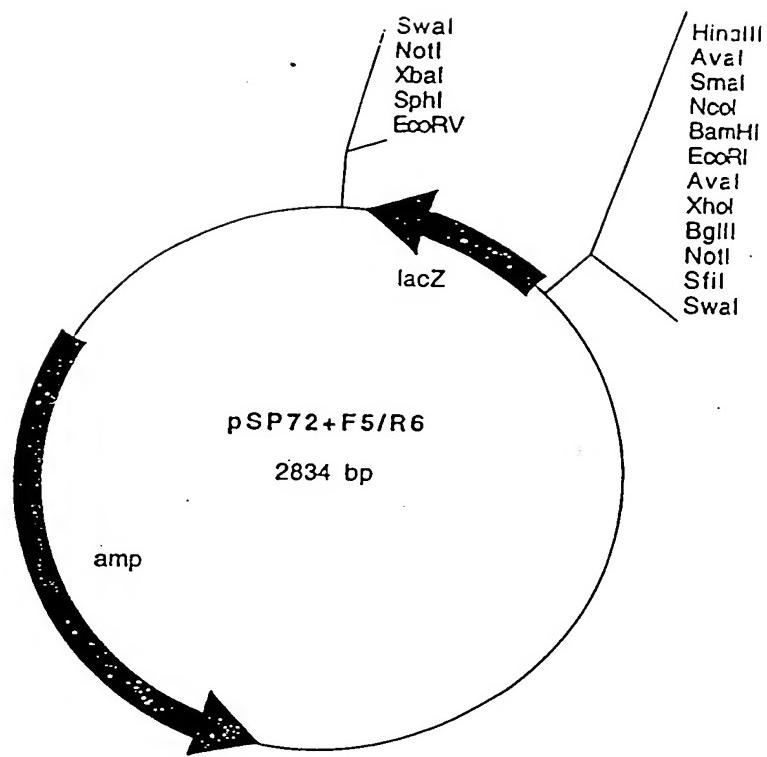
Inhibition of endothelial cell proliferation



pTrc                    pTrcPlatelet Factor-4 (47-70)    pTrcThrombospondin (13-40)

Inhibitory activity of lysates from *Salmonella* strains  
expressing anti-angiogenic peptides

FIG. 20

**FIG. 21**

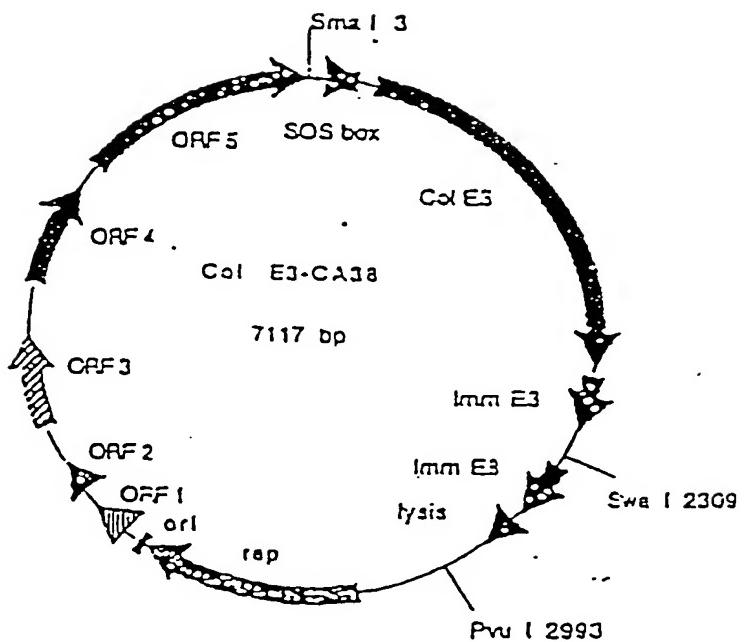


FIG. 22

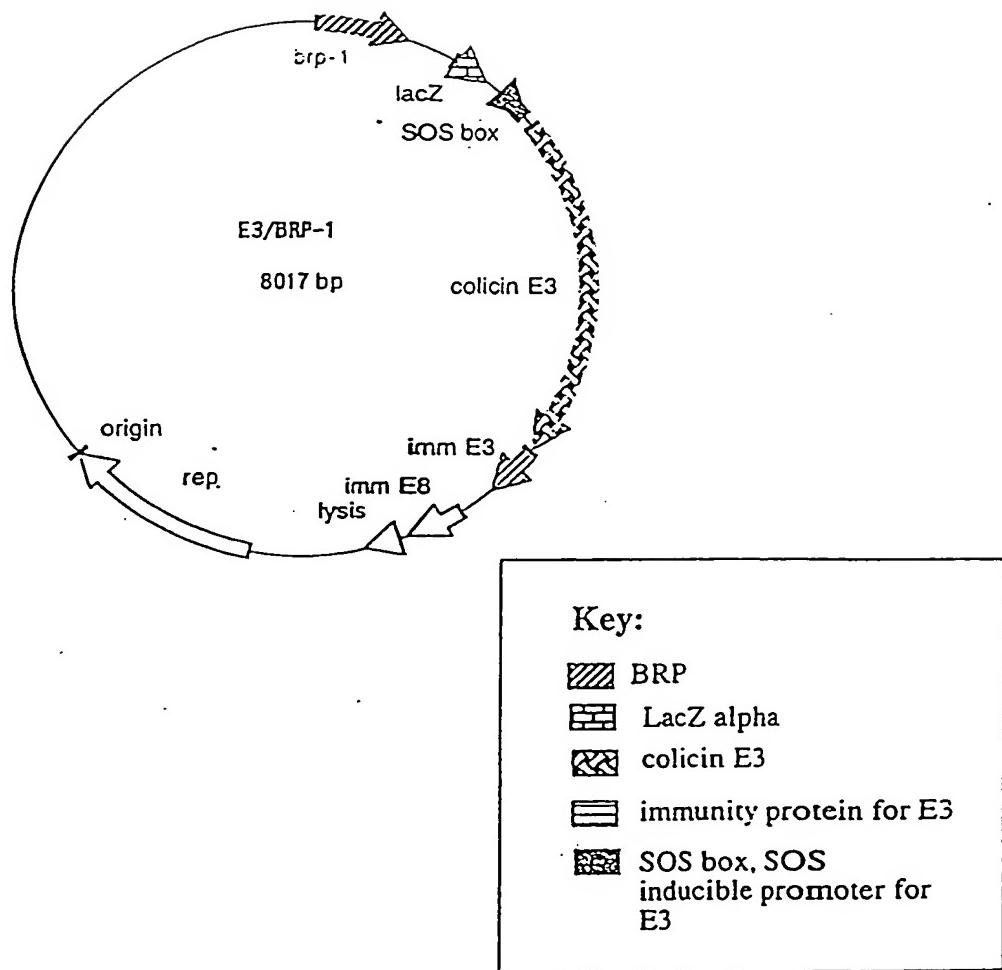
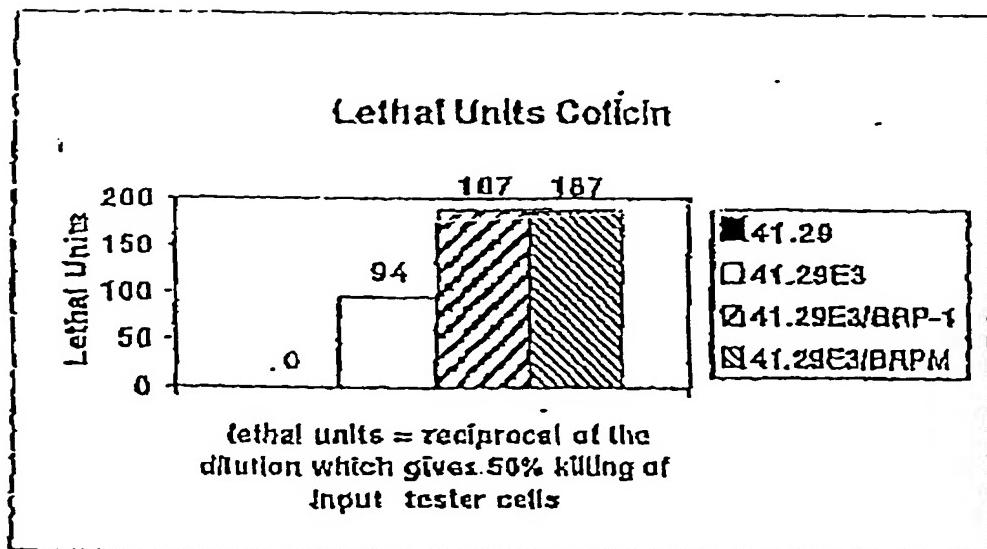
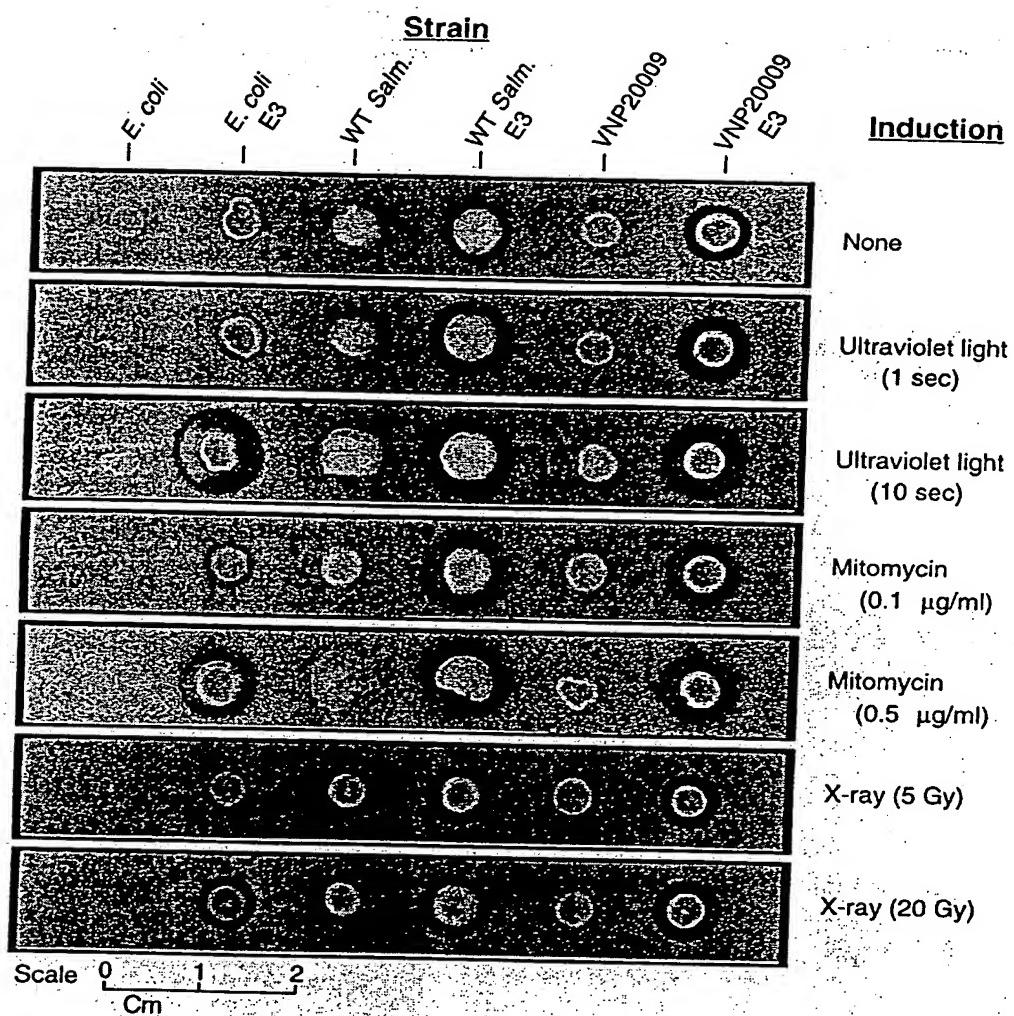


FIG. 23

**FIG. 24**

**FIG. 25**

## Efficacy of 41.2.9/ColE3 on C38 Murine Colon Carcinoma

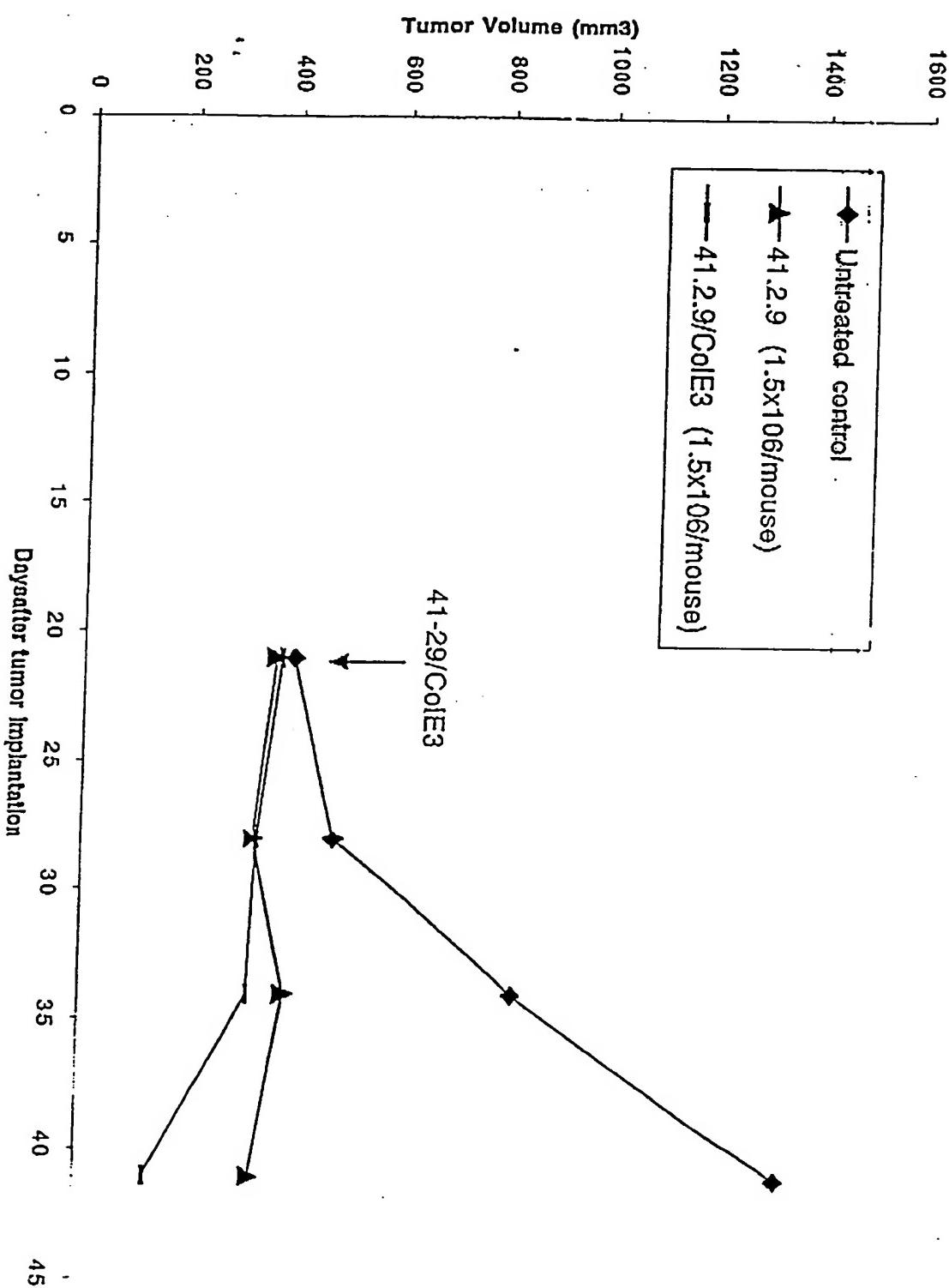
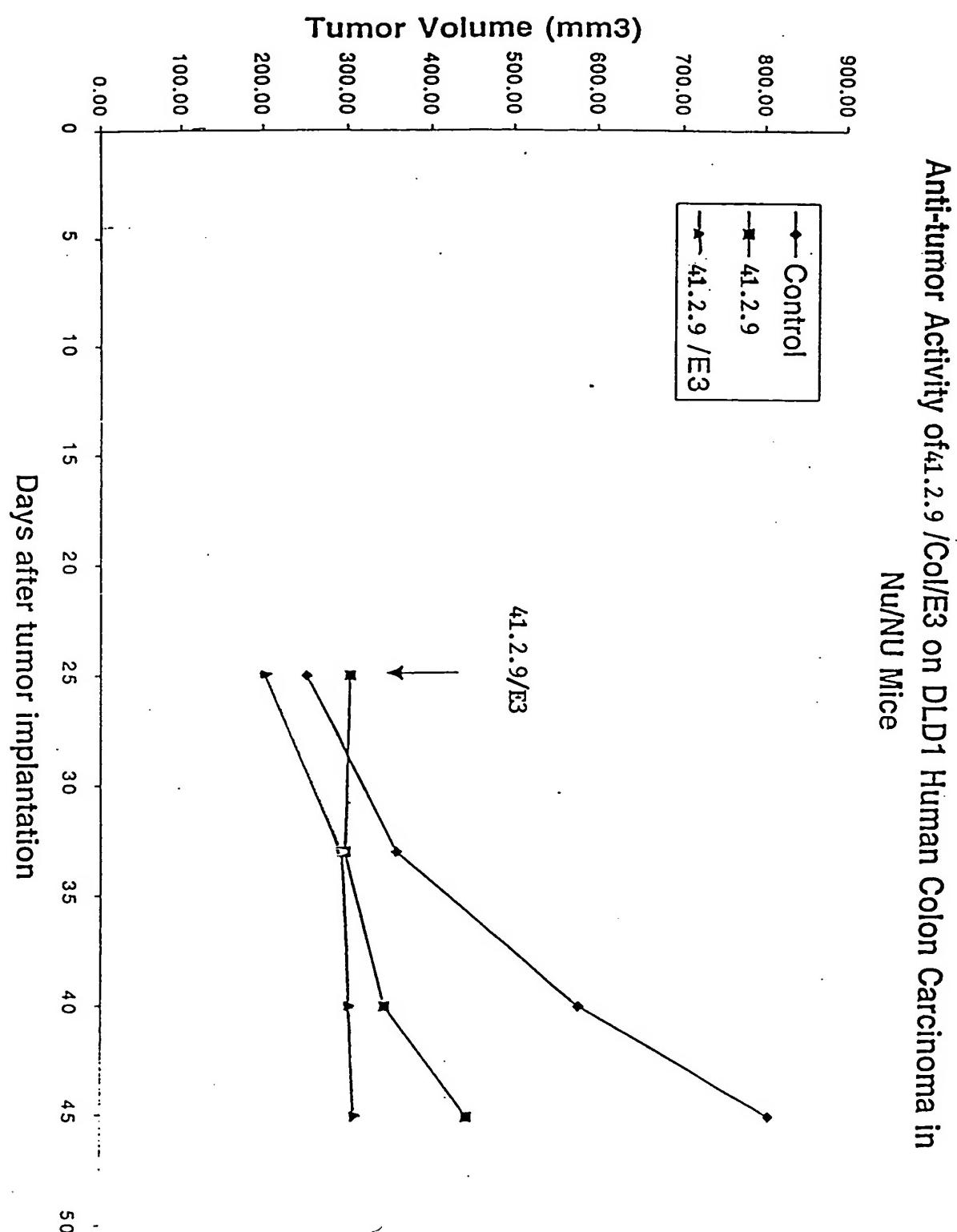


FIG. 26

**FIG. 27**

8002-059

(SHEET 28 OF 41)

Efficacy of 41.2.9/ColE3 on B16 Murine Melanoma

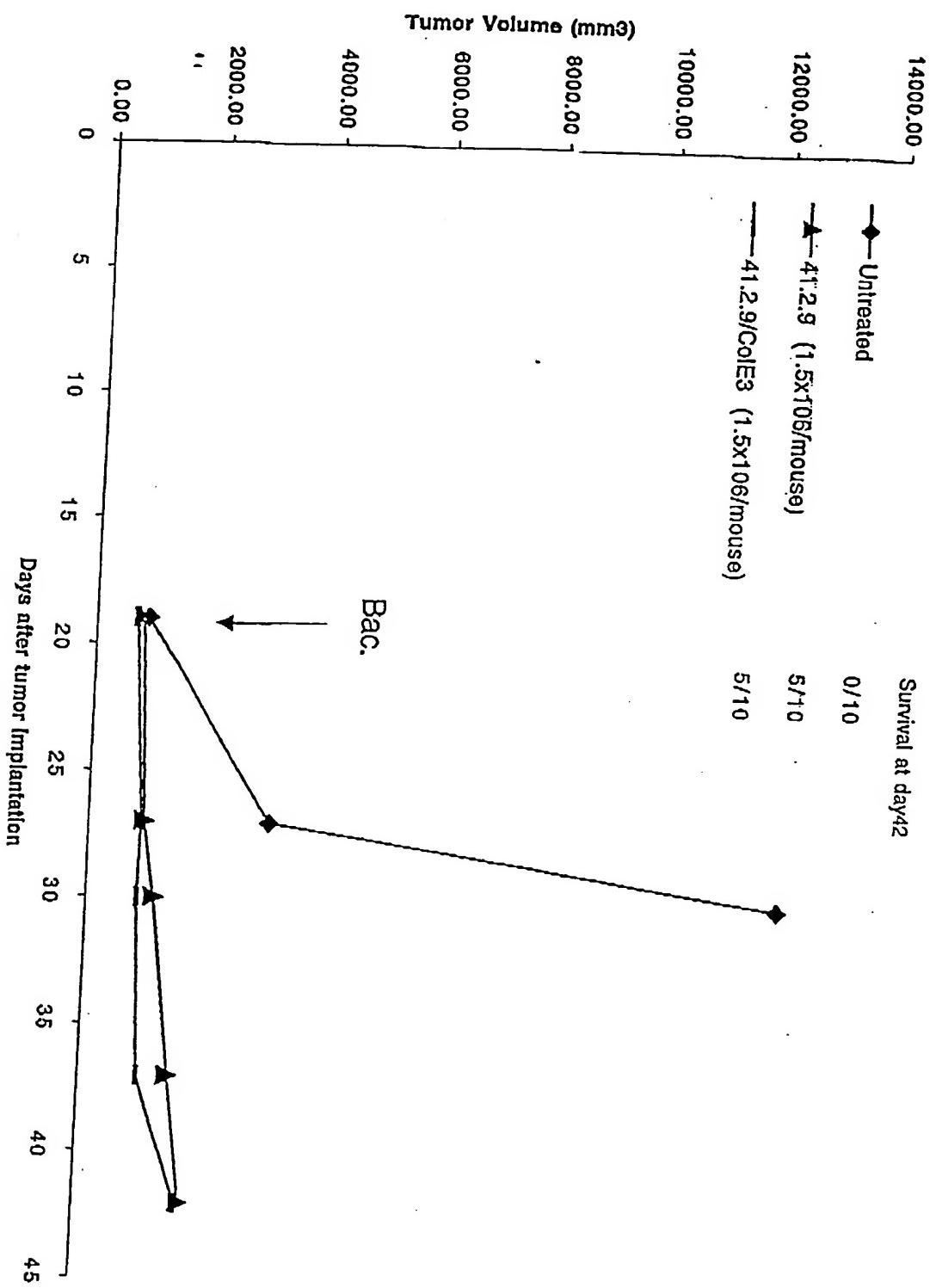
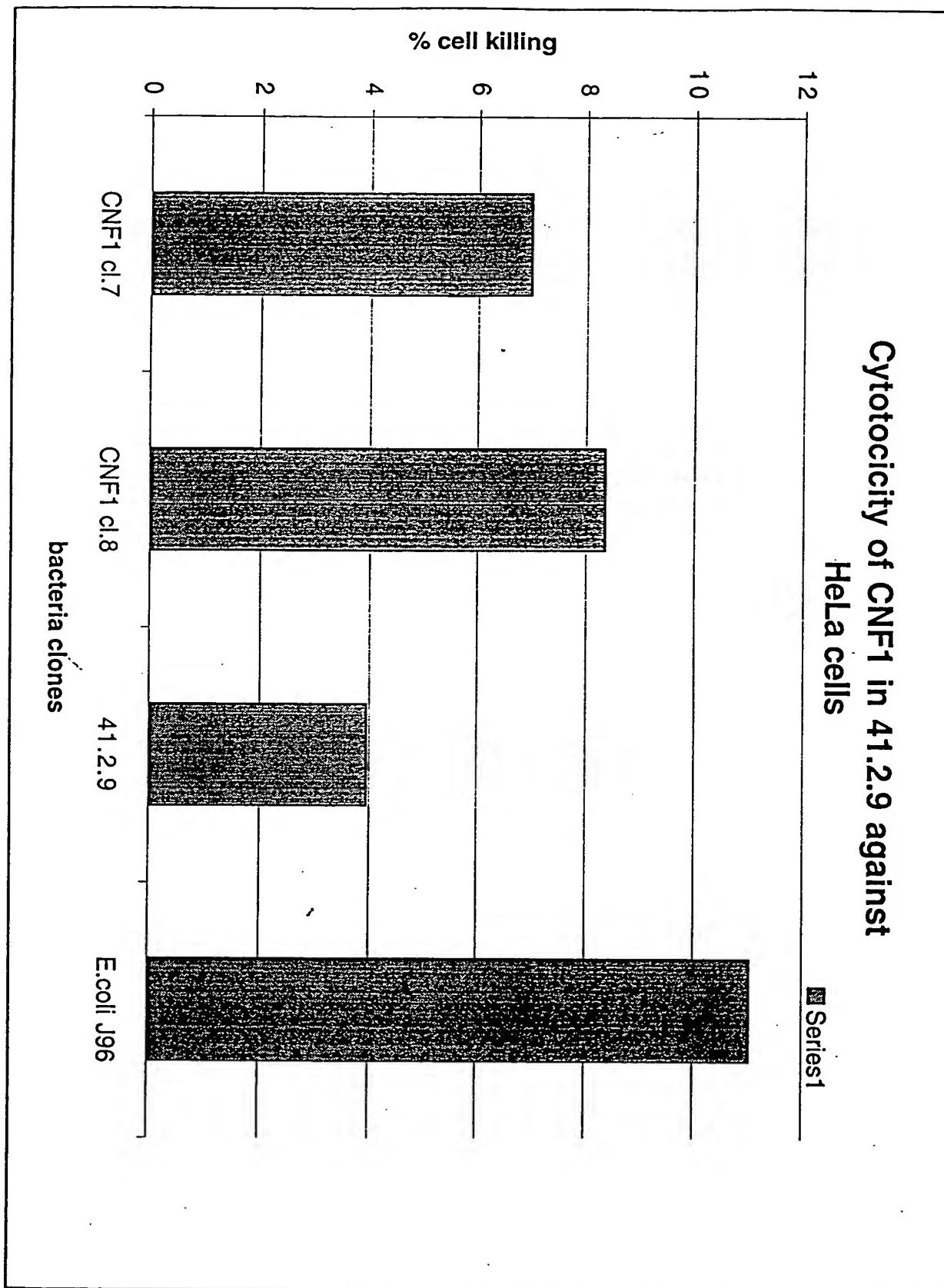
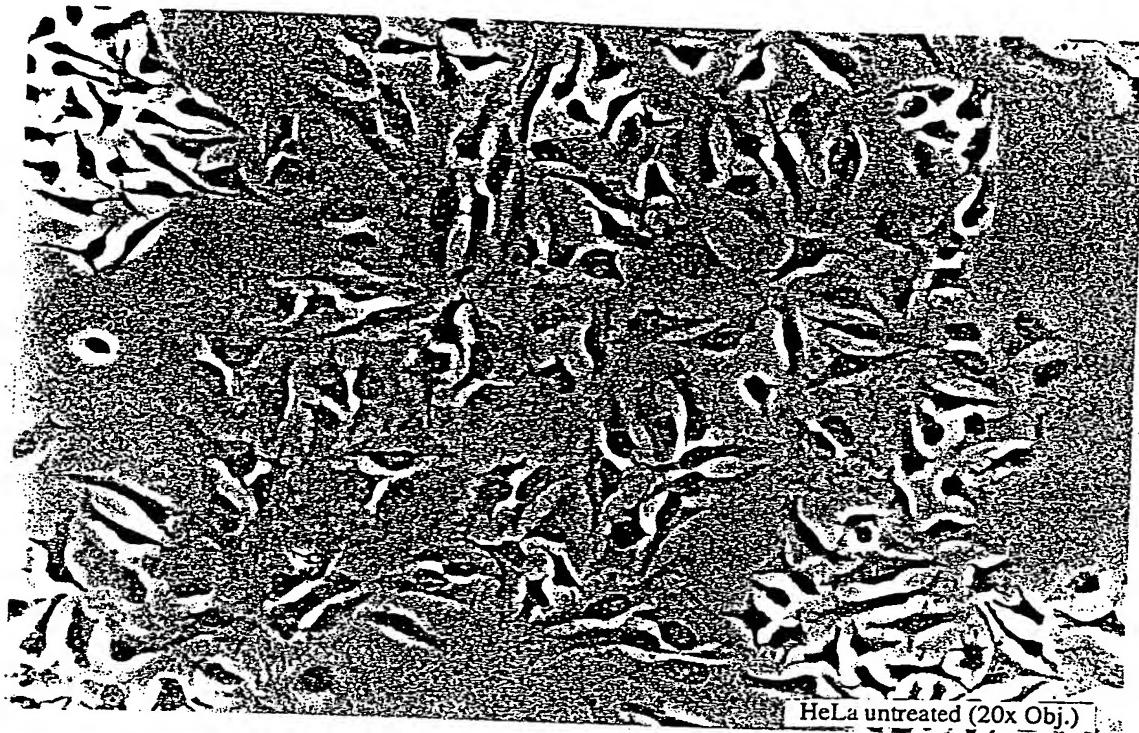
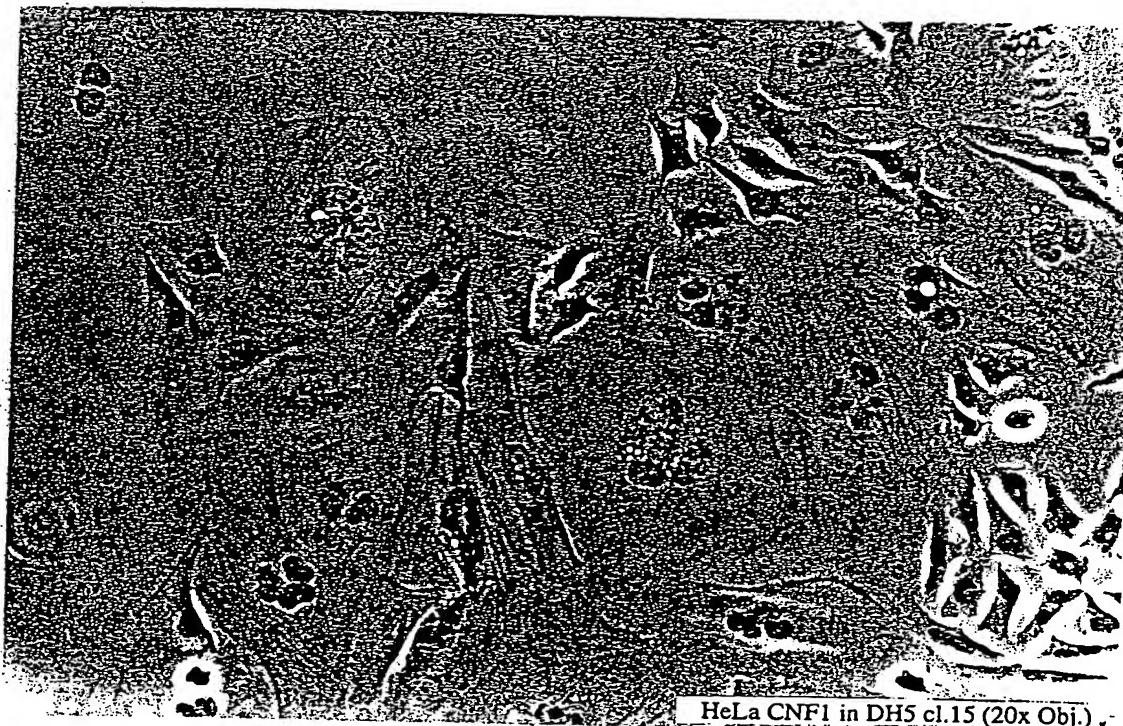


FIG. 28

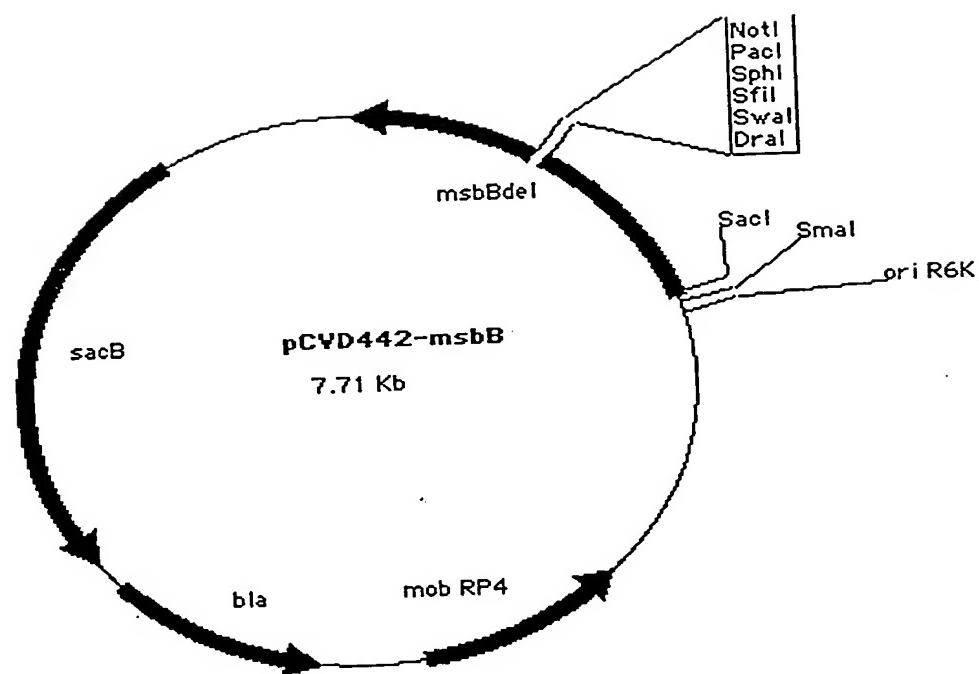


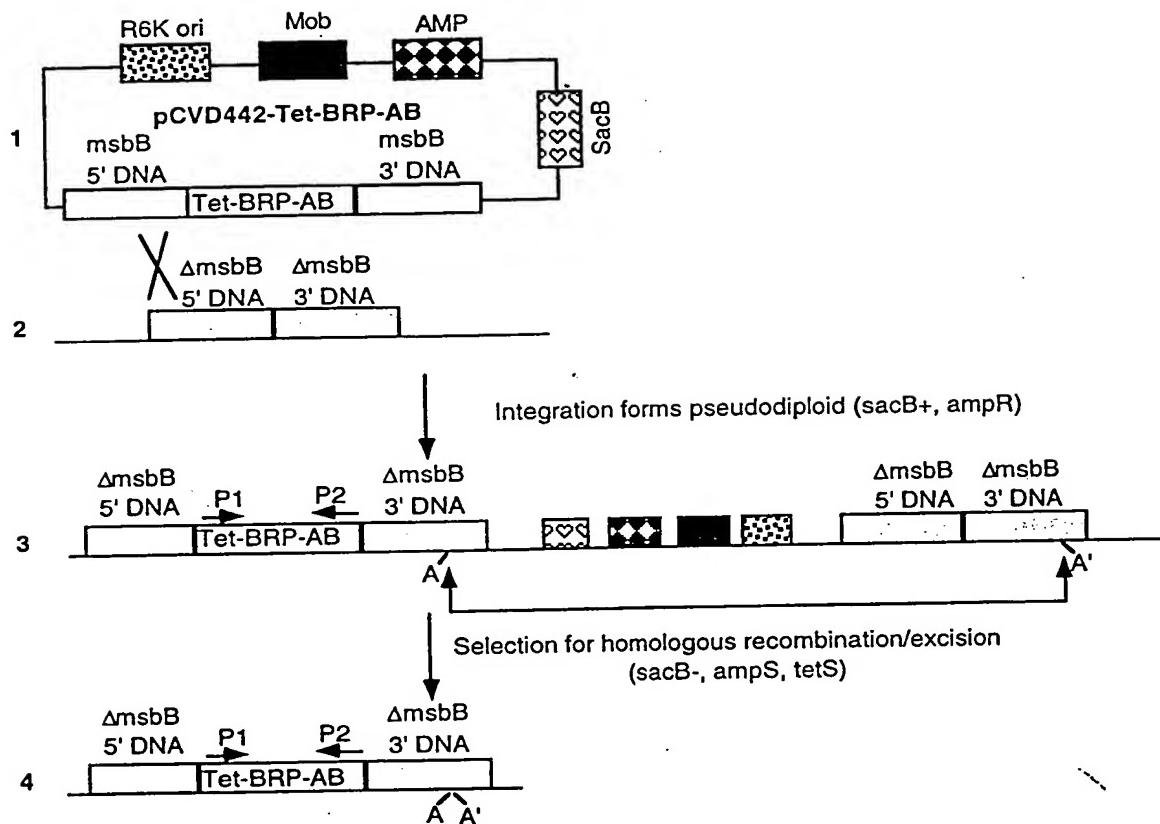
**FIG. 29**

**A****B****FIG. 30**

GATATCATTC	TGGCCTCTGA	CGTTGTGATG	GTCGCACGTS	GCGATCTGGG	CGTTGAAATC	GGCGATCCGG	70
AGCTGGTGG	TATCCAGAAA	GCGCTGATTC	GCCGTGCGCG	TCAGCTAAC	CGCGCAGTCA	TCACCGCAAC	140
GCAAATGATG	GAGTCGATGA	TCACCAACCC	GATGCCGACC	CGTGCAGAAG	TGATGGACGT	GGCGAACGCC	210
GTCTGGATG	GCACGGATGC	GGTTATGCTG	TCTGCCAAA	CCGCAGCCGG	TCAGTATCCT	TCTGAAACCG	280
TTGCCGAAAT	GGCGCGCGTC	TGCCCTGGCG	CAGAAAAAAT	CCCCAGCATC	AATGTGCTA	AACACCGTCT	350
CGACGTGCAG	TTCGACAACG	TTGAAGAAGC	CATTGCCATG	TCTGCGATGT	ATGCGGCAA	CCATCTGAAA	420
GGCGTTACCG	CGATCATCAC	CATGACGGAA	TCCGGTGT	CCCGCCTAAT	GACTTCCCCT	ATCAGCTCCG	490
GCCCTGCCAT	TTTCGCCATG	TGCGGCCATG	AACCGACGCT	GAACCTGACC	GCGCTCTATC	GCGGAGTAAC	560
GCCGGTGCAT	TTTGATAGCG	CGGCTGATGG	CGMTGCGCG	GCACATGAAG	CTGTTAATCT	GCTGCGCGAT	630
AAAGGGTATC	TGGTTTCCCG	CGACCTGGTT	ATCGTACCC	AGGGCGATGT	CATGAGCACC	GTCGGTTCAA	700
CCAATACAC	GCGGCCGCC	CCCTAATTAA	CCCCCATGC	GGGGGGCCAT	ATAGGCCGGG	GATTAAATG	770
CAAACGTCCG	CGGAAACGCC	GACGCACTGT	GTTCCAGATA	TAGTCAAAAA	CCGGATTACC	CTGATTATGA	840
AACATGCCG	CCATTTTTTG	CCCCTAGAGAG	GCCATCAGCA	TGGCTGGAAT	GTCGACGCC	CAGCCATGCG	910
GTACGAGAAA	AATGACTTTT	TCGTCGTTAC	GACCGCATCTC	CTCGATAATC	TCCAGACCTT	CCCAGTCAAC	980
ACGCTGTTGA	ATTTTTTCG	GACCGCGCAT	CGCCAACCTCA	GCCATCATCG	CCATTGCCCTG	TGGCGCGGTG	1050
GCGAACATCT	CATCGACAAT	CGCTTCGCGC	TCAGCTTCGC	TACGCTGCCG	AAAGCACAAC	GACAGATTAA	1120
TTAGCGCCCG	GCGACGAGAA	CTCTTCCCCA	GCCGTCCGGC	AAAACGCC	AGCGTCGCCA	GCAAAGGGTC	1190
GCGGAATGAT	GCGGTGTTA	ATGCGATCCC	CGCCATTGCC	GCCGCGGCCA	ACCAGGGCC	CCAATACTGT	1260
GGATAGCGAA	AGGATTTTTC	GAATTCAAGG	ATATACTCAC	TATTATTTTT	TTTGGTTTCC	ATGCTTTCC	1330
AGGGTCTGCT	GACCGAAGAA	GGAATTGTGA	ATAGTGTAGC	GACGTCTGCC	TCTCACACAA	AACAAAAAAG	1400
CCGGCACACA	TGCGTACCG	GCTCTGTCAG	CGCATTTGTT	AATCGAAGCG	CAGTTGCC	AGAACCTCTT	1470
TCACCTGTGC	CAGGTATTCA	CGACGATCTG	ACCCCGTCAG	ACCTTCCGTG	CGCGGCAATT	TTGCTGTCAG	1530
AGGGTTAACG	GCTTGCTGGT	TGATC					1555

FIG. 31

**FIG. 32**

**FIG.33**

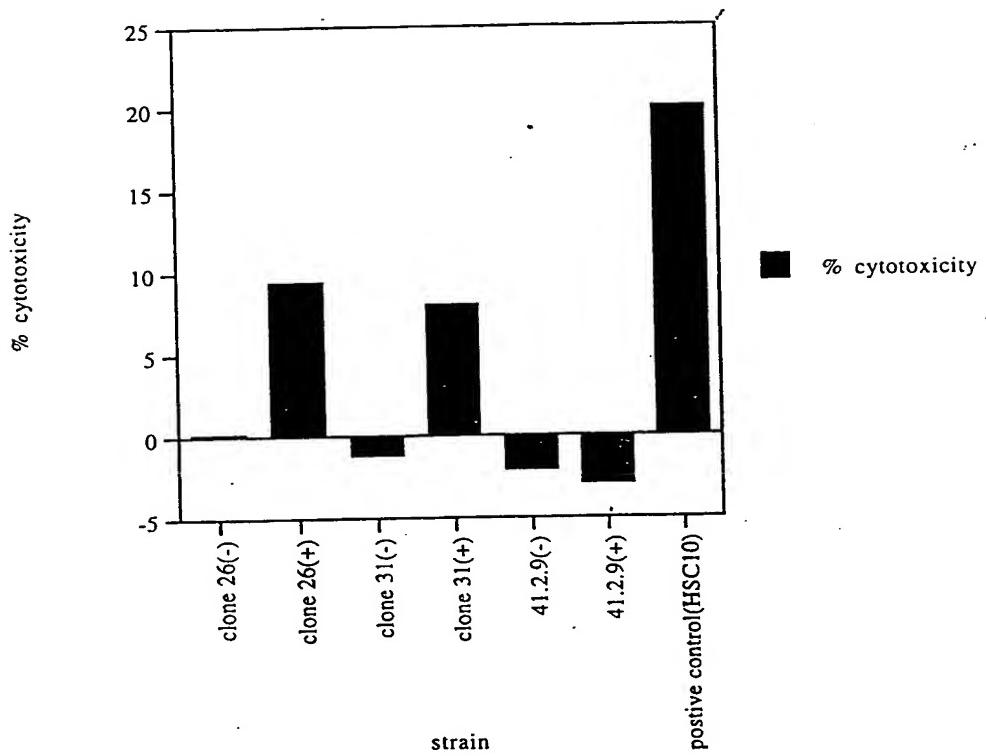
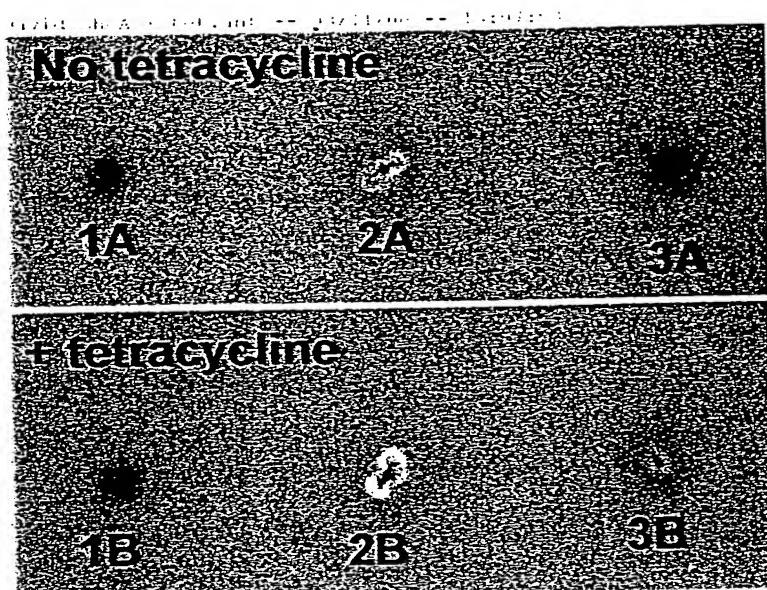


FIG. 34



**FIG. 35**

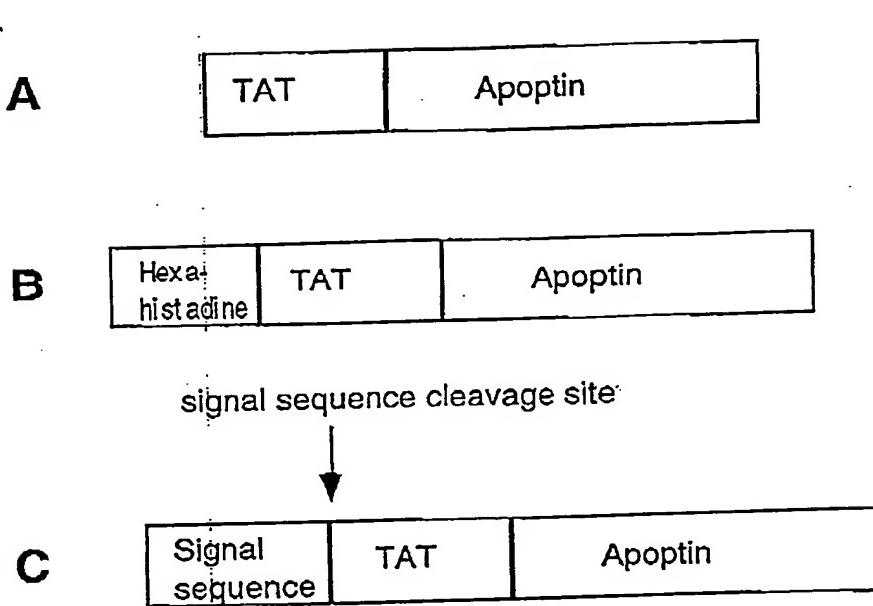


FIG. 36

## Protein Sequence of 616-4 F

page 1

Length of 616-4 F: 551 bp; Listed from: 1 to: 551;  
 Translated from: 7 to: 409 (Entire region);  
 Genetic Code used: Universal; Wed, Aug 16, 2000 1:40 PM

Frame 1

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NAG	ACC	ATG	GCT	TAT	GGC	AGA	AAA	AAA	AGA	AGA	CAG	AGA	AGA	AGA	ATG	AAC
9											36				45	

A	L	Q	E	D	T	P	P	G	P	S	T	V	F	R	P	P	T	S	
GCG	CTG	CAG	GAA	GAT	ACC	CCG	CCG	GGC	CCG	TCC	ACC	GTG	TTT	CGC	CCG	CCG	ACC	TCC	
60						69					87				96			105	

S	R	P	L	E	T	P	H	C	R	E	I	R	I	G	I	A	G	I	
TCC	CGC	CCG	CTG	GAA	ACC	CCG	CAT	TGC	CGC	GAA	ATC	CGC	ATC	GCG	ATC	GCG	GGC	ATC	
117								135			144			153				162	

T	I	T	L	S	L	C	G	C	A	N	A	R	A	P	T	L	R	S	
ACC	ATC	ACC	CTG	TCC	CTG	TGC	GGC	TGC	GGC	AAC	GCG	CGC	GCG	CCG	ACC	CTG	CGC	TCC	
174						183				192			201			210		219	

A	T	A	D	N	S	E	N	T	G	F	K	N	V	P	D	L	R	T	
GCG	ACC	GCG	GAT	AAC	TCC	GAA	AAC	ACC	GCG	TTT	AAA	AAC	GTC	CCG	GAT	CTG	CGC	ACC	
231								240		249			258			267		276	

D	Q	P	K	P	P	S	K	K	R	S	C	D	P	S	E	Y	R	V	
GAT	CAG	CCG	AAA	CCG	CCG	TCC	AAA	AAA	CGC	TCC	TGC	GAT	CCG	TCC	GAA	TAT	CGC	GTC	
288								297		306			315			324		333	

S	E	L	K	E	S	L	I	T	T	T	P	S	R	P	R	T	A	R	
TCC	GAA	CTG	AAA	GAA	TCC	CTG	ATC	ACC	ACC	ACC	CCG	TCC	CGC	CCG	CGC	ACC	GCC	CGC	
345								354		363			372			381		390	

R	C	I	R	L	.	.	.	.	.	.	.	.	.	.	.	.	.	.		
CGC	TGC	ATC	CGC	CTC	TGA	AAG	CTT	GGC	TGT	TTT	GGC	GGG	TGA	GAG	AAG	ATT	TTC	AGC		
402											420			429			438		447	

CTG	ATA	CAG	ATT	AAA	TCA	GAA	CGC	AGA	AGC	GGT	CTG	ATA	AAA	CAG	AAT	TTG	CCT	GGC		
459									468		477			486			495		504	

GGC	AGT	AGC	GCG	GTG	GTC	CCA	CCT	GAC	CCC	ATG	CCG	AAC	TCA	GA	.	.	.	.	
516																			
														525		534		543	

FIG. 37

## Protein Sequence of TAP6H8 trcF

page 1

Length of TAP6H8 trcF: 751 bp; Listed from: 1 to: 444;  
 Translated from: 7 to: 427 (Entire region);  
 Genetic Code used: Universal; Mon, Aug 14, 2000 3:19 PM

Frame 1

M	A	H	H	H	H	H	H	Y	G	R	K	K	R	R	
NAG	ACC	ATG	GCT	CAT	CAC	CAT	CAC	CAC	TAT	GGC	CGC	AAA	AAA	CGC	CGT
9		18		27					36					45	

Q	R	R	R	M	N	A	L	Q	E	D	T	P	P	G	P	S	T	V
CAG	CGC	CGT	CGC	ATG	AAC	GCG	CTG	CAG	GAA	GAT	ACC	CCG	CCG	GGC	CCG	TCC	ACC	GTG
60		69		78					87					96		105		

F	R	P	P	T	S	S	R	P	L	E	T	P	H	C	R	E	I	R
TTT	CGC	CCG	CCG	ACC	TCC	TCC	CGC	CCG	CTG	GAA	ACC	CCG	CAT	TGC	CGC	GAA	ATC	CGC
117		126		135					144					153		162		

I	G	I	A	G	I	T	I	T	L	S	L	C	G	C	A	N	A	R
ATC	GGC	ATC	GCG	GCG	ATC	ACC	ATC	ACC	CTG	TCC	CTG	TGC	GGC	TGC	GCG	AAC	GCG	CGC
174		183		192					201					210		219		

A	P	T	L	R	S	A	T	A	D	N	S	E	N	T	G	F	K	N
GCG	CCG	ACC	CTG	CGC	TCC	GCG	ACC	GCG	GAT	AAC	TCC	GAA	AAC	ACC	GGC	TTT	AAA	AAC
231		240		249					258					267		276		

V	P	D	L	R	T	D	Q	P	K	P	P	S	K	K	R	S	C	D
GTC	CCG	GAT	CTG	CGC	ACC	GAT	CAG	CCG	AAA	CCG	CCG	TCC	AAA	AAA	CGC	TCC	TGC	GAT
288		297		306					315					324		333		

P	S	E	Y	R	V	S	E	L	K	E	S	L	I	T	T	T	P	S
CCG	TCC	GAA	TAT	CGC	GTC	TCC	GAA	CTG	AAA	GAA	TCC	CTG	ATC	ACC	ACC	ACC	CCG	TCC
345		354		363					372					381		390		

R	P	R	T	A	R	R	C	I	R	L	.							
CGC	CCG	CGC	ACC	GCC	CGC	CGC	TGC	ATC	CGC	CTC	TGA	AAG	CTT	GGC	TGT	TTT		
402		411		420					429					438				

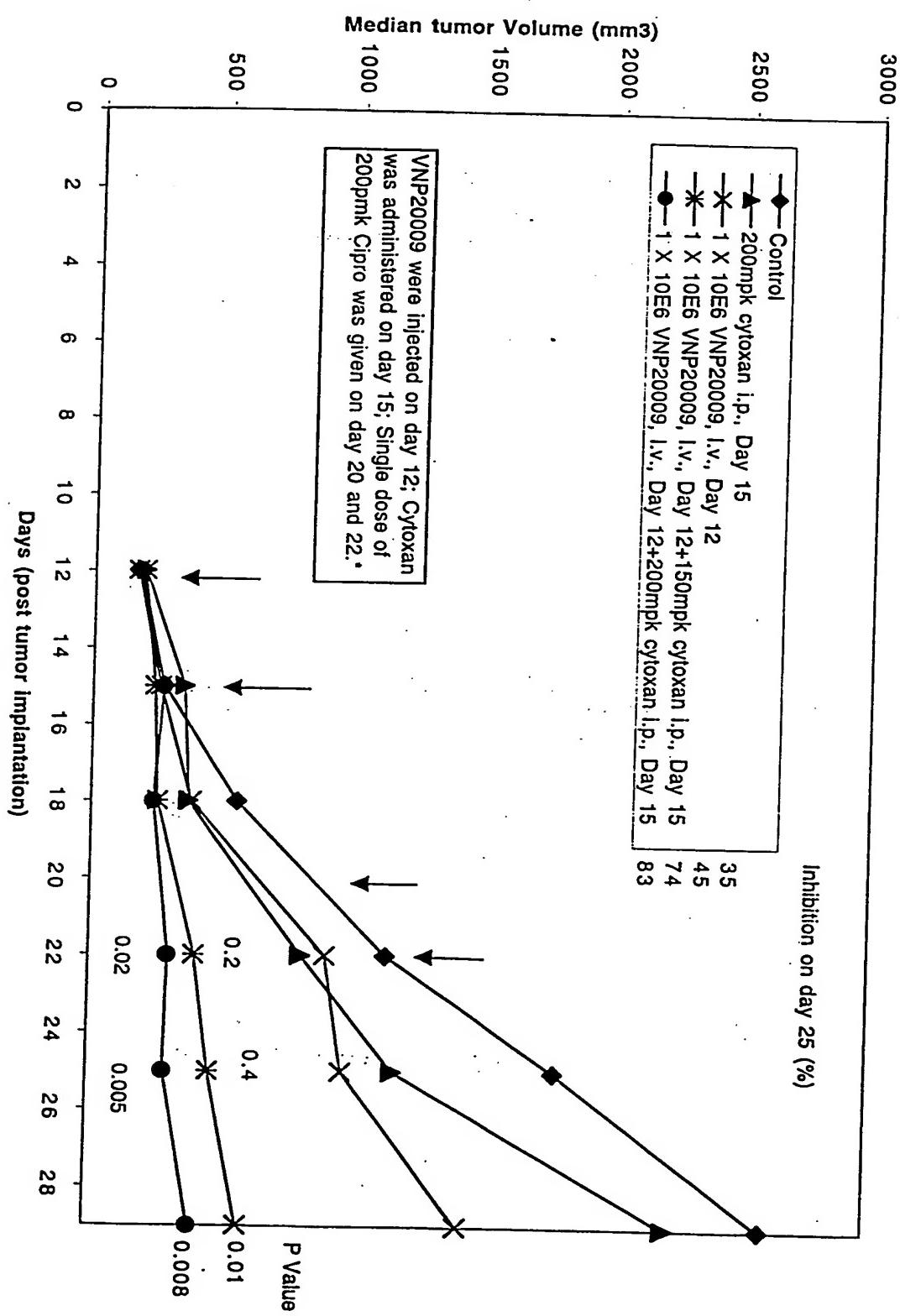


FIG. 39

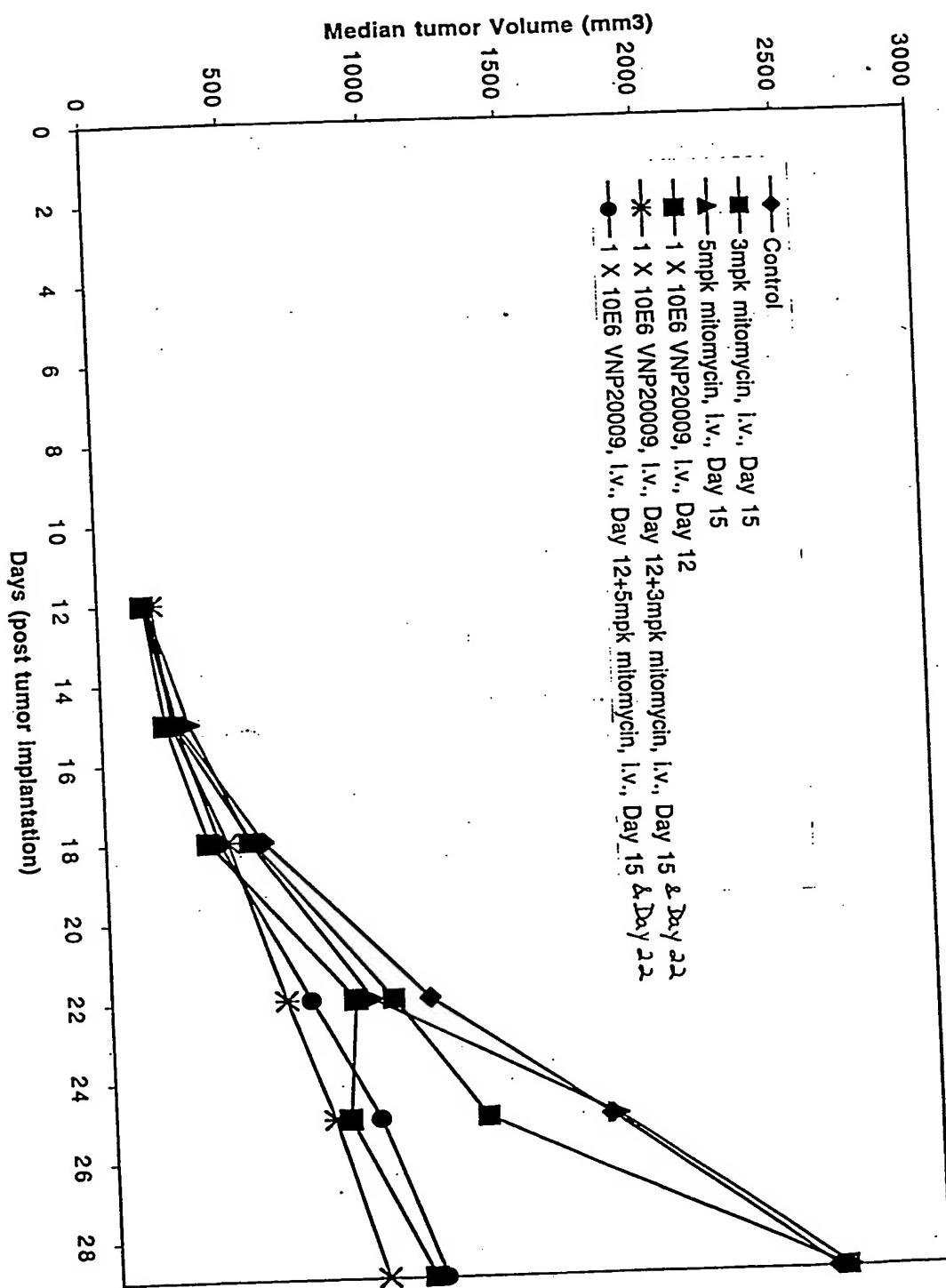


FIG. 40

8002-059

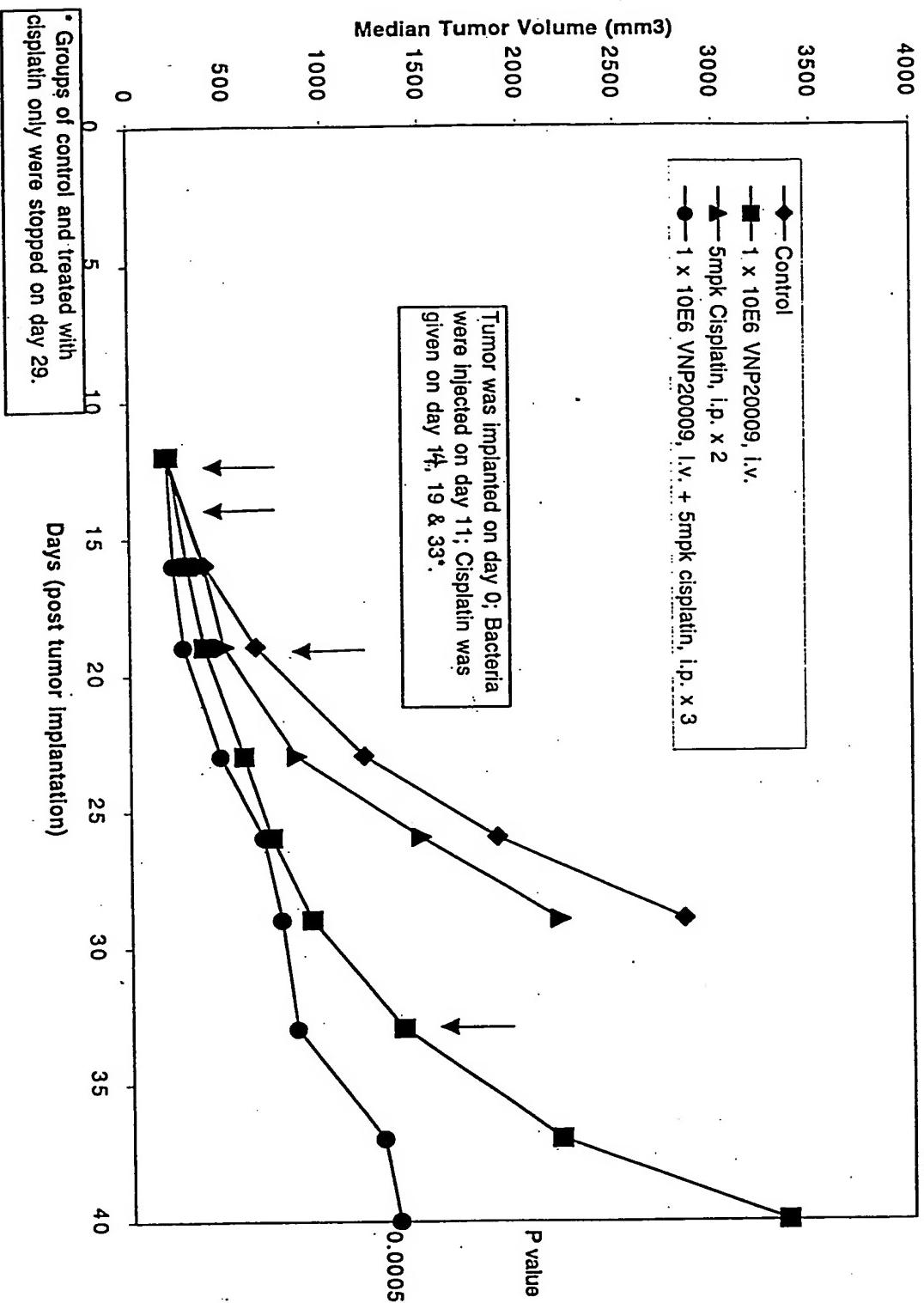


FIG. 41